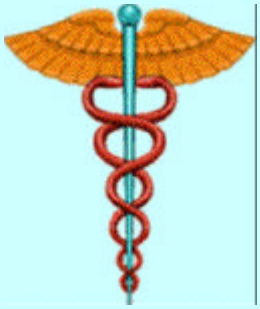
MEDICAL MANAGEMENT

OF BIOLOGICAL CASUALTIES



HANDBOOK

U.S. ARMY MEDICAL RESEARCH

INSTITUTE OF INFECTIOUS DISEASES

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MEDICAL MANAGEMENT

OF BIOLOGICAL CASUALTIES

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DISCLAIMER

The purpose of this Handbook is to provide concise supplemental reading material to assist in education of biological casualty management. Every effort has been made to make the information in this handbook consistent with official policy and doctrine. The information contained in this handbook is **not** official Department of the Army policy or doctrine, and it should not be construed as such.

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INTRODUCTION

Medical defense against biological warfare is an area of study for military health care providers which does not apply readily to the day to day mission of caring for patients in peacetime. However, during Operations Desert Shield/Desert Storm, it became obvious that the threat of biological attacks against our soldiers was real, and that we could do more to educate our medical professionals about how to prevent and treat biological warfare casualties. Many of our medical personnel who deployed for the Gulf War had less than an optimal understanding of the biological threat and of the medical means available to counter it. Since Desert Storm, there has been a renewed emphasis placed on making sure that our health care professionals gain the necessary background in this important area of military medicine.

In fact, our training efforts have significantly intensified over the past eighteen months following increased incidents and threats of domestic terrorism (e.g., New York City

World Trade Center bombing, Tokyo subway sarin release, Oklahoma City federal building bombing, Atlanta Centennial Park bombing). Additionally, the recent escalation of tensions in Iraq and subsequent deployment of military troops to the Persian Gulf region underscored the importance of force protection from biological threats. The Secretary of Defense announced in November 1997 that all U.S. military troops will be immunized against anthrax. Finally, the disclosure of a sophisticated offensive biological warfare program in the Former Soviet Union (FSU) and subsequent media attention has reinforced the need for increased training and education.

The Medical Management of Chemical and Biological Casualties Course taught at both USAMRIID and USAMRICD was revised in March 1998 by doubling its class capacity providing education in both biological and chemical medical defense to over 560 military medical professionals per calendar year. Also, the highly successful 3-day USAMRIID satellite course on the Medical Management of Biological Casualties presented in September 1997 reached over 5600 military and other government health care professionals throughout the United States.

Through this handbook and the training courses noted above, military medical professionals will learn that effective medical countermeasures are available against many of the bacteria, viruses, and toxins which might be used as biological weapons against our military forces. The importance of this education cannot be overemphasized and it is hoped that our physicians, nurses, and allied medical professionals will develop a solid understanding of the biological threats we face and the medical armamentarium for defending against these threats.

The global biological warfare threat is taken seriously by our leaders. The United States was willing to return to war against Iraq in February 1998 to preserve the integrity and the independence of the UNSCOM inspectors such that they would have unconditional, unfettered and unrestricted access to all suspected sites in Iraq in their search for weapons of mass destruction. The threat is indeed serious, and the potential for devastating casualties is high for certain biological agents. However, with appropriate use of medical countermeasures either already developed or under development, many casualties can be prevented or minimized, and the fighting strength of our forces can be maintained.

The purpose for this handbook is to serve as a small and concise manual for medical personnel to carry in their BDU pocket as a guide to medical prophylaxis and management of biological casualties. It is designed as a quick reference and overview, and is not intended as a definitive text on the medical management of biological casualties.

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HISTORY OF BIOLOGICAL WARFARE

AND CURRENT THREAT

The use of biological weapons and efforts to make them more useful as a means of waging war have been recorded numerous times in history. Two of the earliest reported uses occurred in the 6th century BC, with the Assyrians poisoning enemy wells with rye ergot, and Solon's use of the purgative herb hellebore during the siege of Krissa. In 1346, plague broke out in the Tartar army during its siege of Kaffa (at present day Feodosia in Crimea). The attackers hurled the corpses of those who died over the city walls; the plague epidemic that followed forced the defenders to surrender, and some infected people who left Kaffa may have started the Black Death pandemic which spread throughout Europe. Russian troops may have used the same plague-infected corpse tactic against Sweden in 1710.

On several occasions, smallpox was used as a biological weapon. Pizarro is said to have presented South American natives with variola-contaminated clothing in the 15th century, and the English did the same when Sir Jeffery Amherst provided Indians loyal to the French with smallpox-laden blankets during the French and Indian War of 1754 to 1767. Native Americans defending Fort Carillon sustained epidemic casualties which directly contributed to the loss of the fort to the English.

In this century, there is evidence that during World War I, German agents inoculated horses and cattle with glanders in the U.S. before the animals were shipped to France. In 1937, Japan started an ambitious biological warfare program, located 40 miles south of Harbin, Manchuria, in a laboratory complex code named "Unit 731". Studies directed by Japanese General Ishii continued there until 1945, when the complex was leveled by burning it. A post World War II investigation revealed that numerous organisms had received Japanese research attention, and that experiments had been conducted on prisoners of war. Slightly less than 1,000 human autopsies apparently were carried out at Unit 731, most on victims exposed to aerosolized anthrax. Many more prisoners and Chinese nationals may have died in this facility - some have estimated up to 3,000 human deaths. In 1940, a plague epidemic in China and Manchuria followed reported overflights by Japanese planes dropping plague-infected fleas. By 1945, the Japanese program had stockpiled 400 kilograms of anthrax to be used in a specially designed fragmentation bomb.

In 1943, the United States began research into the offensive use of biological agents. This work was started, interestingly enough, in response to a perceived German biological warfare (BW) threat as opposed to a Japanese one. The United States conducted this research at Camp Detrick (now Fort Detrick), which was a small National Guard airfield prior to that time, and produced agents at other sites until 1969, when President Nixon stopped all offensive biological and toxin weapon research and production by executive order. Between May 1971 and May 1972, all stockpiles of biological agents and munitions from the now defunct U.S. program were destroyed in the presence of monitors representing the United States Department of Agriculture, the Department of Health, Education, and Welfare, and the states of Arkansas, Colorado, and Maryland. Included among the destroyed agents were *Bacillus anthracis*, botulinum

toxin, *Francisella tularensis*, *Coxiella burnetii*, Venezuelan equine encephalitis virus, *Brucella suis*, and Staphylococcal enterotoxin B. The United States also had a medical defensive program, begun in 1953, that continues today at USAMRIID.

In 1972, the United States and many other countries signed the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction, commonly called the Biological Weapons Convention. This treaty prohibits the stockpiling of biological agents for offensive military purposes, and also forbids research into such offensive employment of biological agents. The former Soviet Union and the government of Iraq were both signatories to this accord. However, despite this historic agreement among nations, biological warfare research continued to flourish in many countries hostile to the United States. There were also several cases of suspected or actual use of biological weapons. Among the most notorious of these were the "yellow rain" incidents in Southeast Asia, the accidental release of anthrax at Sverdlovsk, and the use of ricin as an assassination weapon in London in 1978.

Testimony from the late 1970's indicated that the countries of Laos and Kampuchea were attacked by planes and helicopters delivering aerosols of several colors. After being exposed, people and animals became disoriented and ill, and a small percentage of those stricken died. Some of these clouds were thought to be comprised of trichothecene toxins (in particular, T2 mycotoxin). These attacks are lumped under the label "Yellow Rain". There has been a great deal of controversy about whether these clouds were truly biological warfare agents: some have argued that the clouds were nothing more than bee feces produced by swarms of bees.

In late April of 1979, an incident occurred in Sverdlovsk (now Yekaterinburg) in the former Soviet Union which appeared to be an accidental release of anthrax in aerosol form from the Soviet Military Compound 19, a microbiology facility. Residents living downwind from this compound developed high fever and difficulty breathing, and a large number died. The final death toll was estimated at the time to be between 200 and 1,000. The Soviet Ministry of Health blamed the deaths on the consumption of contaminated meat, and for years controversy raged in the press over the actual cause of the outbreak. All evidence available to the United States government indicated a massive release of aerosolized anthrax. In the summer of 1992, U.S. intelligence officials were proven correct when new Russian President Boris Yeltsin acknowledged that the Sverdlovsk incident was in fact a large scale accident involving the escape of an aerosol of anthrax spores from the military research facility. In 1994, Meselson and colleagues published an in-depth analysis of the Sverdlovsk incident (*Science* 266:1202-1208). They documented that all of the 1979 cases occurred within a narrow zone extending downwind in a southerly direction from Compound 19. A total of 77 patients were identified by Meselson's team, including 66 fatalities and 11 survivors.

Before the Sverdlovsk incident, in 1978, a Bulgarian exile named Georgi Markov was attacked in London with a device disguised as an umbrella which injected a tiny pellet filled with ricin toxin into the subcutaneous tissue of his leg while he was waiting for a

bus. He died several days later. On autopsy, the tiny pellet was found and determined to contain the toxin. This assassination, it was later revealed, was carried out by the communist Bulgarian government, and the technology to commit the crime was supplied to the Bulgarians by the former Soviet Union.

In August of 1991, the first United Nations inspection of Iraq's biological warfare capabilities was carried out in the aftermath of the Gulf War. On August 2, 1991, representatives of the Iraqi government announced to leaders of United Nations Special Commission Team 7 that they had conducted research into the offensive use of *Bacillus anthracis*, botulinum toxins, and *Clostridium perfringens* (presumably one of its toxins). This was the first open admission of biological weapons research by any country in recent memory, and it verified many of the concerns of the U.S. intelligence community publicly. Iraq had extensive and redundant research facilities at Salman Pak and other sites, many of which were destroyed during the war.

In 1995, further information on Iraq's offensive program was made available to United Nations inspectors. Iraq conducted research and development work on anthrax, botulinum toxins, *Clostridium perfringens*, aflatoxins, wheat cover smut, and ricin. Field trials were conducted with *Bacillus subtilis* (a simulant for anthrax), botulinum toxin, and aflatoxin. Biological agents were tested in various delivery systems, including rockets, aerial bombs, and spray tanks. In December 1990, the Iraqis filled 100 R400 bombs with botulinum toxin, 50 with anthrax, and 16 with aflatoxin. In addition, 13 Al Hussein (SCUD) warheads were filled with botulinum toxin, 10 with anthrax, and 2 with aflatoxin. These weapons were deployed in January 1991 to four locations. All in all, Iraq produced 19,000 liters of concentrated botulinum toxin (nearly 10,000 liters filled into munitions), 8,500 liters of concentrated anthrax (6,500 liters filled into munitions) and 2,200 liters of aflatoxin (1,580 liters filled into munitions).

The threat of biological warfare has increased in the last two decades, with a number of countries working on offensive use of these agents. The extensive program of the former Soviet Union is now controlled largely by Russia. Russian president Boris Yeltsin has stated that he will put an end to further offensive biological research; however, the degree to which the program has been scaled back, if any, is not known. Recent revelations from a senior BW program manager who defected from the FSU in 1992 outlined a remarkably robust biological warfare program including active research into genetic engineering, binary biologicals and chimeras. There is also growing concern that the smallpox virus, eliminated from the face of the earth in the late 1970's and now stored in only two laboratories at the CDC in Atlanta and the Institute for Viral Precautions in Moscow, Russia, may have been "bargained" away by desperate Russian scientists seeking money.

There is intense concern in the West about the possibility of proliferation or enhancement of offensive programs in countries hostile to the western democracies, due to the potential hiring of expatriate Russian scientists. It was reported in January 1998 that Iraq had sent about a dozen scientists involved in BW research to Libya to help that country develop a biological warfare complex disguised as a medical facility in

the Tripoli area. In a report issued in November 1997, Secretary of Defense William Cohen singled out Libya, Iraq, Iran, and Syria as countries "aggressively seeking" nuclear, biological, and chemical weapons.

There is also an increasing amount of concern over the possibility of terrorist use of biological agents to threaten either military or civilian populations. There have been cases of persons loyal to extremist groups trying to obtain microorganisms which could be used as biological weapons. The Department of Defense is leading a federal effort to train the first responders in 120 American cities to be prepared to act in case of a domestic terrorist incident involving WMD.

Certainly the threat of biological weapons being used against U.S. military forces is broader and more likely in various geographic scenarios than at any point in our history. Therefore, awareness of this potential threat and education of our leaders and medical care providers on how to combat it are crucial.

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MEDICAL ASPECTS OF THE BIOLOGICAL THREAT

Many bacteria, fungi, viruses, rickettsial agents, and toxins have been mentioned in various literature sources as possible biological warfare agents. Those mentioned most often include *Bacillus anthracis* (anthrax), botulinum toxin, *Yersinia pestis* (plague), ricin, Staphylococcal enterotoxin B (SEB), and Venezuelan equine encephalitis virus (VEE). Despite the very different characteristics of these organisms, viruses, and toxins, biological agents used as weapons share some common characteristics. They can be dispersed in aerosols of particle size one to five micrometers (microns), which may remain suspended (in certain weather conditions) for hours and if inhaled will penetrate into distal bronchioles and terminal alveoli of victims. Particles larger than five microns would tend to be filtered out in the upper airway. The aerosols may be delivered by simple technology, including industrial sprayers with nozzles modified to generate the smaller particle size. The aerosol could be delivered from a line source such as an airplane or boat traveling upwind of the intended target, or from a point source such as a stationary sprayer or missile dispensing agent-containing bomblets in an area upwind of the target. The weather in the target area is very important in the employment of biological agents as aerosols, as higher wind speeds tend to break up the aerosol cloud, and stable wind direction is obviously important. Inversion conditions and lower wind speeds, 5 to 10 miles per hour, conditions which occur more often during nighttime and early morning hours, would be ideal for dispensing such aerosols. Other possible routes of exposure for biological agents include oral, by intentional contamination of food and water, and percutaneous. In general, these other routes of exposure are considered less important than the respiratory route.

Diseases produced by the offensive use of biological agents against U.S. forces could be lethal and/or disabling. From a military standpoint, incapacitation of a high percentage of friendly forces may be as operationally significant as effects caused by more lethal agents. Examples of lethal agents include *Bacillus anthracis*, botulinum toxin, and *Francisella tularensis*, while incapacitating agents include SEB and *Coxiella burnetii*. Some agents, such as *Yersinia pestis* and *C. burnetii*, would produce pulmonary syndromes characteristic of the endemic disease they produce in nature. Others, such as botulinum toxin, although delivered by a different route of exposure (respiratory) than usual with endemic disease, would produce a similar clinical picture to that commonly seen with oral exposure. Person-to-person spread could be important for some agents, such as smallpox and pneumonic plague, and local disease cycles might occur if a competent vector for a bacterium or virus is present in the environment (e.g., fleas for *Y. pestis* and certain mosquitoes for Venezuelan equine encephalitis).

The potential impact of biological weapons is well illustrated by a World Health Organization publication from 1970 (Health Aspects of Chemical and Biological Weapons, WHO, 1970). It was estimated that fifty kilograms of aerosolized *B. anthracis* spores, for example, dispensed by a line source 2 kilometers upwind of a population center of 500,000 unprotected people in ideal meteorological conditions, would travel greater than 20 kilometers downwind, and kill/incapacitate up to 125,000 people in the path of the biological cloud. If *F. tularensis* was dispensed, the number of dead/incapacitated was estimated to be about 125,000. Thus, if properly employed as offensive weapons under ideal meteorological conditions, certain biological organisms could truly be weapons of mass destruction.

In addition to their detrimental health effects on the targeted population, biological warfare agents would likely cause significant impacts on the medical care system. Overwhelming numbers of patients, and demands for intensive care would overwhelm medical resources. Special medications or vaccines not generally available in standard pharmaceutical stocks would be required. Medical care providers and laboratory personnel might need added protection, and autopsy and interment of remains could present hazards not commonly dealt with.

The medical response to the threat or use of biological weapons may be different depending on whether medical measures are employed prior to exposure, or whether exposure has already occurred and/or symptoms are present. If provided before exposure, active immunization or prophylaxis with antibiotics may prevent illness in those exposed. Active immunization may be effective against several potential biological warfare agents, and is probably the best modality for future protection of U.S. military forces against a wide variety of biological threats. After exposure, active or passive immunization as well as pre-treatment with therapeutic antibiotics or antiviral drugs may ameliorate disease symptoms. After onset of illness, only diagnosis of the disease and general or specific treatment are left to medical care providers. The good news is that excellent vaccines and antitoxins exist for several of the most likely biological warfare agents, and more are under development.

BACTERIAL AGENTS

Bacteria are unicellular organisms. They vary in shape and size from spherical cells - cocci - with a diameter of 0.5-1.0 μ m (micrometer), to long rod-shaped organisms - bacilli - which may be from 1-5 μ m in size. Chains of bacilli may exceed 50 μ m. The shape of the bacterial cell is determined by the rigid cell wall. The interior of the cell contains the nuclear material (DNA), cytoplasm, and cell membrane, that are necessary for the life of the bacterium. Many bacteria also have glycoproteins on their outer surfaces which aid in bacterial attachment to surface receptors on cells and are of special importance in their ability to cause disease. Under special circumstances some types of bacteria can transform into spores. The spore of the bacterial cell is more resistant to cold, heat, drying, chemicals and radiation than the bacterium itself. Spores are a dormant form of the bacterium and, like the seeds of plants, they can germinate when conditions are favorable.

Bacteria can cause diseases in human beings and animals by means of two mechanisms which differ in principle: in one case by invading the tissues, in the other by producing poisons (toxins). In many cases pathogenic bacteria possess both properties. The diseases they produce often respond to specific therapy with antibiotics. This manual will cover several of the bacteria or rickettsia considered to be potential BW threat agents: *Bacillus anthracis* (Anthrax), *Brucella* spp. (Brucellosis), *Vibrio cholerae* (Cholera), *Burkholderia mallei* (Glanders), *Yersinia pestis* (Plague), *Francisella tularensis* (Tularemia), and *Coxiella burnetii* (Q Fever).

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ANTHRAX

SUMMARY

Signs and Symptoms: Incubation period is 1-6 days. Fever, malaise, fatigue, cough and mild chest discomfort is followed by severe respiratory distress with dyspnea, diaphoresis, stridor, and cyanosis. Shock and death occurs within 24-36 hours after onset of severe symptoms.

Diagnosis: Physical findings are non-specific. A widened mediastinum may be seen on CXR. Detectable by Gram stain of the blood and by blood culture late in the course of illness.

Treatment: Although effectiveness may be limited after symptoms are present, high dose antibiotic treatment with penicillin, ciprofloxacin, or doxycycline should be undertaken. Supportive therapy may be necessary.

Prophylaxis: An FDA licensed vaccine is available. Vaccine schedule is 0.5 ml SC at 0, 2, 4 weeks, then 6, 12, and 18 months for the primary series, followed by annual boosters. Oral ciprofloxacin or doxycycline for known or imminent exposure.

Isolation and Decontamination: Standard precautions for healthcare workers. After an invasive procedure or autopsy is performed, the instruments and area used should be thoroughly disinfected with a sporicidal agent (chlorine).

OVERVIEW

Bacillus anthracis, the causative agent of Anthrax, is a rod-shaped, gram-positive, sporulating organism with the spores constituting the usual infective form. Anthrax is primarily a zoonotic disease of herbivores, with cattle, sheep and horses being the usual domesticated animal hosts, but other animals may be infected. Human disease may be contracted by handling contaminated hair, wool, hides, flesh, blood and excreta of infected animals and from manufactured products such as bone meal, as well as by purposeful dissemination of spores. Infection is introduced through scratches or abrasions of the skin, wounds, inhalation of spores, eating insufficiently cooked infected meat, or by flies. All human populations are susceptible. Recovery from an attack of the disease may be followed by immunity. The spores are very stable and may remain viable for many years in soil and water. They will resist sunlight for varying periods.

HISTORY AND SIGNIFICANCE

Anthrax spores were weaponized by the United States in the 1950's and 1960's before the old U.S. offensive program was terminated. Other countries have weaponized this agent or are suspected of doing so. The anthrax bacterium is easy to cultivate and spore production is readily induced. Spores are highly resistant to sunlight, heat and disinfectants - properties which could be advantageous when choosing a bacterial weapon. Iraq admitted to a United Nations inspection team in August of 1991 that it had performed research on the offensive use of *B. anthracis* prior to the Persian Gulf War of 1991, and in 1995 Iraq admitted to weaponizing anthrax. This agent could be produced in either a wet or dried form, stabilized for weaponization by an adversary and delivered as an aerosol cloud either from a line source such as an aircraft flying upwind of friendly positions, or as a point source from a spray device. Coverage of a large ground area could also be theoretically facilitated by multiple spray bomblets disseminated from a missile warhead at a predetermined height above the ground.

CLINICAL FEATURES

Anthrax presents as three distinct clinical syndromes in man: cutaneous, inhalational, and gastrointestinal disease. The cutaneous form (also referred to as malignant pustule) occurs most frequently on the hands and forearms of persons working with infected livestock. It begins with a papule followed by formation of a blister-like fluid-filled vesicle.

The vesicle typically dries and forms a coal-black scab, hence the term anthrax (Greek for coal). Sometimes this local infection will develop into a systemic infection which is often fatal. Endemic inhalational anthrax, known as Woolsorters' disease, is a rare infection contracted by inhalation of the spores. It occurs mainly among workers handling infected hides, wool, and furs. The intestinal form, which is also very rare in man, is contracted by the ingestion of insufficiently cooked meat from infected animals. In man, the mortality of untreated cutaneous anthrax ranges up to 25 per cent; in inhalational and intestinal cases, the case fatality rate is almost 100 percent.

DIAGNOSIS

After an incubation period of 1-6 days, presumably dependent upon the dose and strain of inhaled organisms, the onset of inhalation anthrax is gradual and nonspecific. Fever, malaise, and fatigue may be present, sometimes in association with a nonproductive cough and mild chest discomfort. These initial symptoms are often followed by a short period of improvement (hours to 2-3 days), followed by the abrupt development of severe respiratory distress with dyspnea, diaphoresis, stridor, and cyanosis. Shock and death usually follow within 24-36 hours after the onset of respiratory distress. Physical findings are typically non-specific. The chest X-ray may reveal a widened mediastinum ± pleural effusions late in the disease in about 55% of the cases, but typically is without infiltrates. *Bacillus anthracis* will be detectable by Gram stain of the blood and by blood culture with routine media, but often not until late in the course of the illness. Only vegetative encapsulated bacilli are present during infection. Spores are not found within the body unless it is open to ambient air. Studies of inhalation anthrax in non-human primates (rhesus monkey) showed that bacilli and toxin appear in the blood late on day 2 or early on day 3 post-exposure. Toxin production parallels the appearance of bacilli in the blood and tests are available to rapidly detect the toxin. Concurrently with the appearance of anthrax, the WBC count becomes elevated and remains so until death.

MEDICAL MANAGEMENT

Almost all inhalational anthrax cases in which treatment was begun after patients were significantly symptomatic have been fatal, regardless of treatment. Penicillin has been regarded as the treatment of choice, with 2 million units given intravenously every 2 hours. Tetracyclines and erythromycin have been recommended in penicillin allergic patients. The vast majority of naturally-occurring anthrax strains are sensitive *in vitro* to penicillin. However, penicillin-resistant strains exist naturally, and one has been recovered from a fatal human case. Moreover, it might not be difficult for an adversary to induce resistance to penicillin, tetracyclines, erythromycin, and many other antibiotics through laboratory manipulation of organisms. All naturally occurring strains tested to date have been sensitive to erythromycin, chloramphenicol, gentamicin, and ciprofloxacin. In the absence of information concerning antibiotic sensitivity, treatment should be instituted at the earliest signs of disease with intravenous ciprofloxacin (400 mg q 8-12 hrs) or intravenous doxycycline (200 mg initially, followed by 100 mg q 12 hrs). Supportive therapy for shock, fluid volume deficit, and adequacy of airway may all be needed.

Standard Precautions should be practiced. After an invasive procedure or autopsy, the instruments and area used should be thoroughly disinfected with a sporicidal agent. Iodine can be used, but must be used at disinfectant strengths, as antiseptic-strength iodophors are not usually sporicidal. Chlorine, in the form of sodium or calcium hypochlorite, can also be used, but with the caution that the activity of hypochlorites is greatly reduced in the presence of organic material.

PROPHYLAXIS

Vaccine: A licensed vaccine is derived from sterile culture fluid supernatant taken from an attenuated strain. The vaccination series consists of six 0.5 ml doses SC at 0, 2, and 4 weeks, then 6, 12 and 18 months, followed by yearly boosters. Limited human data suggest that the vaccine protects against cutaneous anthrax. There is insufficient data to know its efficacy against inhalational anthrax in humans, although studies in rhesus monkeys indicate that good protection can be afforded after only two doses (15 days apart) for up to 2 years. However, it should be emphasized that the vaccine series should be completed according to the routine 6 dose primary series. As with all vaccines, the degree of protection depends upon the magnitude of the challenge dose; vaccine-induced protection could presumably be overwhelmed by extremely high spore challenge. Restart the primary vaccine series only if greater than two years elapses between the first and second doses. For all other missed doses, administer the missed dose ASAP and continue the series based on the most current dose.

Contraindications for use of this vaccine include hypersensitivity reaction to a previous dose of vaccine and age < 18 or > 65. Reasons for temporary deferment of the vaccine include pregnancy; active infection with fever; or a course of immune suppressing drugs such as steroids. Reactogenicity is mild to moderate. Up to 6 percent of recipients will experience mild discomfort at the inoculation site for up to 72 hours (e.g., tenderness, erythema, edema, pruritus), while less than 1 percent will experience more severe local reactions, potentially limiting use of the arm for 1-2 days. Modest systemic reactions (e.g., myalgia, malaise, low-grade fever) are uncommon, and severe systemic reactions such as anaphylaxis, which precludes additional vaccination, are rare. The vaccine should be stored between 2-6 ^{o}C (refrigerator temperature, not frozen).

Antibiotics: The choice of antibiotics for prophylaxis is difficult to make; for example, it seems relatively easy to induce penicillin and tetracycline resistance in the laboratory. Therefore, prophylaxis with ciprofloxacin (500 mg po bid) or doxycycline (100 mg po bid) is recommended. If personnel are unvaccinated, a single 0.5 ml dose of vaccine should also be given subcutaneously. Should the attack be confirmed as anthrax, antibiotics should be continued for at least 4 weeks in all those exposed, and until all those exposed have received three doses of the vaccine. Two additional 0.5 ml doses of vaccine should be given 2 weeks apart in the unvaccinated; those previously vaccinated with fewer than three doses should receive a single 0.5 ml booster, while vaccination probably is not necessary for those who have received the initial three-doses of the primary series, within the previous six months. Upon discontinuation of antibiotics, patients should be closely observed; if clinical signs of anthrax occur,

patients should be treated as indicated above. If vaccine is not available, antibiotics should be continued beyond 4 weeks and withdrawn under medical observation. Optimally, patients should have medical care available upon discontinuation of antibiotics, from a fixed medical care facility with intensive care capabilities and infectious disease consultants.

Brucellosis

Summary

Signs and Symptoms: Incubation period from 5-60 days; average of 1-2 months. Highly variable. Acute and subacute brucellosis are non-specific. Irregular fever, headache, profound weakness and fatigue, chills, sweating, arthralgias, mylagias. Depression and mental status changes. Osteoarticular findings (i.e., sacroiliitis, vertebral osteomyleitis). Fatalities are uncommon.

Diagnosis: Blood cultures require a prolonged period of incubation in the acute phase. Bone marrow cultures produce a higher yield. Confirmation requires phage-typing, oxidative metabolism, or genotyping procedures. ELISA's followed by Western blotting are used.

Treatment: Doxycycline and rifampin for a minimum of six weeks. Ofloxacin + rifampin is also effective. Therapy with rifampin, a tetracycline, and an aminoglycoside is indicated for infections with complications such as endocarditis or meningoencephalitis.

Prophylaxis: No approved human vaccine is available. Avoid consumption of unpasteurized milk and cheese.

Isolation and Decontamination: Standard precautions for healthcare workers. Person-to-person transmission via tissue transplantation and sexual contact have been reported but are insignificant. Environmental decontamination can be accomplished with a 0.5% hypochlorite solution.

Overview

The Brucellae are a group of gram-negative cocco-baccillary organisms, of which four species are pathogenic in humans. Abattoir and laboratory worker infections suggest that *Brucella* spp. are highly infectious via the aerosol route. It is estimated that inhalation of only 10 to 100 bacteria is sufficient to cause disease in man. The relatively long and variable incubation period (5-60 days) and the fact that many infections are asymptomatic under natural conditions has made it a less desirable agent for weaponization, although large aerosol doses may shorten the incubation period and increase the clinical attack rate. Brucellosis infection has a low mortality rate (5% of untreated cases) with most deaths caused by endocarditis or meningitis. It is an incapacitating and disabling disease in its natural form.

History and Significance

Marston described disease caused by *B. melitensis* among British soldiers on Malta during the Crimean War as "Mediterranean gastric remittent fever". Work by the Mediterranean Fever Commission identified goats as the source of human brucella infection on Malta, and restriction of the ingestion of unpasteurized goats milk and cheese soon decreased the number of cases of brucellosis among military personnel.

In 1997, most cases were associated with ingestion of unpasteurized dairy products and abattoir and veterinary work. In the United States most cases are reported from Florida, California, Virginia, and Texas. It is a rare disease in the United States with an incidence of 0.5 per 100,000 population.

In 1954, *Brucella suis* became the first agent weaponized by the U.S. in the days of its offensive BW program at the newly constructed Pine Bluff Arsenal. Despite this, *B. melitensis* actually produces more severe human disease.

Clinical Features

Brucellosis may present as a nonspecific febrile illness which resembles influenza. Fever, headache, myalgia, arthralgia, back pain, sweats, chills, and generalized weakness and malaise are common complaints. Cough and pleuritic chest pain may occur in up to twenty percent of cases, but these are usually not associated with acute pneumonitis. Pulmonary symptoms may not correlate with radiographic findings. The chest x-ray may be normal, or show lung abscesses, single or miliary nodules, bronchopneumonia, enlarged hilar lymph nodes, and pleural effusions. Gastrointestinal symptoms occur in up to 70 percent of adult cases, and less frequently in children. These include anorexia, nausea, vomiting, diarrhea and constipation. Ileitis, colitis and granulomatous or a mononuclear infiltrative hepatitis may occur. Lumbar pain and tenderness can occur in up to 60% of cases and is due to various osteoarticular infections of the axial skeletal system. Paravertebral abscesses may occur and can be imaged by CT scan or MRI. CT scans often show vertebral sclerosis. Vertebral and disc space destruction may occur in chronic cases. One or, less frequently, both sacroiliac joints may be infected causing low back and buttock pain that is intensified by stressing the sacroiliac joints on physical exam. Hepatomegaly and splenomegaly can occur in up to 45-63 percent of cases. Peripheral joint involvement may vary from pain on range of motion testing to joint immobility and effusion. Peripheral joint effusions usually show a mononuclear cell predominance and organisms can be isolated in up to 50% of cases. The hip joints are the most commonly involved peripheral joints, but ankle, knee, and sternoclavicular joint infection may occur. Plain radiographs of involved sacroiliac joints usually show blurring of articular margins and widening of the joint space. Technetium or Gallium-67 bone scans are 90% sensitive for detecting sacroileitis and will also detect other sites of bone and joint involvement; they are also useful for differentiating sacroiliac from hip joint involvement.

Meningitis occurs in less than 5% of cases and may be an acute presenting illness of a chronic syndrome occurring late in the course of a persistent infection. The cerebrospinal fluid contains an increased number of lymphocytes and a low to normal glucose. Culture of the CSF has sensitivity of 50%, and specific brucella antibodies can be detected in the fluid in a higher percentage of cases. Encephalitis, peripheral neuropathy, radiculoneuropathy and meningovascular syndromes have also been observed in rare cases. Behavioral disturbances in children and psychoses may occur in the meningoencephalitic form of the disease. Epididymo-orchitis may occur in men as the most frequent genitourinary form of brucellosis. Rashes occur in less than 5% if cases and include macules, papules, ulcers, purpura, petechiae, and erythema nodosum.

Diagnosis

The leukocyte count is usually normal but may be low. Anemia and thrombocytopenia may occur. Blood and bone marrow culture during the acute febrile phase of the illness will yield a positivity rate of 15-70% and 92% respectively. A biphasic culture method for blood (Castaneda bottle) may increase the number of isolates. The serum agglutination test (SAT) will detect both IgM and IgG antibodies. A titer of 1:160 or greater is indicative of active disease. The IgM titer can be measured by adding a reduced agent such as 2-mercaptoethanol to the serum. This will destroy the agglutinability of IgM allowing the IgM titer to be measured by subtracting the now lower titer from the total serum agglutinin titer. A dot-ELISA using an autoclaved extract of *B. abortus* has been found to be a sensitive and specific screening test for detection of *Brucella* antibodies under field conditions. ELISA tests for antibody detection require standardization using a specific antigen before they will be widely available. Antigen detection on DNA extracted from blood mononuclear cells has been accomplished using PCR analysis of a target sequence on the 31-kilodalton *B. abortus* protein BCSP 31. This test has been proven to be rapid and specific and may replace blood culture in the future, since the latter may require incubation for up to 6 weeks. PCR for *Brucella* species is not available at this time except in research laboratories, but shows promise for future use.

Medical Management

Isolation is not required other than contact isolation for draining lesions. Person to person transmission is possible via contact with such lesions. Biosafety level 3 practices should be used for suspected brucella cultures in the laboratory because of the danger of inhalation infection. Antibiotic therapy is recommended as the sole therapy unless there are surgical indications for the treatment of localized diseases (e.g., valve replacement for endocarditis).

The treatment recommended by the World Health Organization for acute brucellosis in adults is doxycycline 200 mg/day p.o. plus rifampin 600-900 mg/day for a minimum of six weeks. The previously established regimen of intramuscular streptomycin along with an oral tetracycline may give fewer relapses but is no longer the primary recommendation. Ofloxacin 400 mg/day and rifampin 600 mg/day p.o. is also an

effective combination. Combination therapy with rifampin, a tetracycline, and an aminoglycoside is indicated for infections with complications such as meningoencephalitis or endocarditis. Doxycycline clearance is increased in the presence of rifampin and plasma levels are lower than when streptomycin is used instead of rifampin.

Prophylaxis

Live animal vaccines are used widely. Consumption of unpasteurized milk and cheese should be avoided. No approved human brucella vaccine is available. An experimental human brucellosis vaccine has been tested on 271 subjects with a 25% rate of unpleasant acute side effects, but no long term adverse side effects.

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Cholera

Summary

Signs and Symptoms: Incubation period 4 hours to 5 days; average 2-3 days. Asymptomatic to severe with sudden onset. Vomiting, headache, intestinal cramping with little or no fever followed rapidly by painless, voluminous diarrhea. Fluid losses may exceed 5 to 10 liters per day. Without treatment, death may result from severe dehydration, hypovolemia and shock.

Diagnosis: Clinical diagnosis. ‘Rice water’ diarrhea and dehydration. Microscopic exam of stool samples reveals few or no red or white cells. Can be identified by darkfield or phase contrast microscopy, and by direct visualization of darting motile vibrio.

Treatment: Fluid and electrolyte replacement. Antibiotics (tetracycline, ciprofloxacin or erythromycin) may shorten the duration of diarrhea and, more importantly, reduce shedding of the organism.

Prophylaxis: A licensed, killed vaccine is available but provides only about 50 percent protection that lasts for no more than 6 months. Vaccination schedule is at 0 and 4 weeks, with booster doses every 6 months.

Isolation and Decontamination: Standard Precautions for healthcare workers. Personal contact rarely causes infection; however, enteric precautions and careful hand-washing should be employed. Bactericidal solutions (hypochlorite) would provide adequate decontamination.

OVERVIEW

Vibrio cholerae is a short, curved, motile, gram-negative, non-sporulating rod. There are two serogroups, O1 and O139, that have been associated with cholera in humans. The O1 serotype exists as 2 biotypes, classical and El Tor. The organisms are facultative anaerobes, growing best at a pH of 7.0, but able to tolerate an alkaline environment. They do not invade the intestinal mucosa, but rather "adhere" to it. Cholera is the prototype toxigenic diarrhea, which is secretory in nature. All strains elaborate the same enterotoxin, a protein molecule with a molecular weight of 84,000 daltons. The entire clinical syndrome is caused by the action of the toxin on the intestinal epithelial cell. Fluid loss in cholera originates in the small intestine with the colon being relatively insensitive to the toxin. The large volume of fluid produced in the upper intestine overwhelms the capacity of the lower intestine to absorb. Transmission is made through direct or indirect fecal contamination of water or foods, and by heavily soiled hands or utensils. All populations are susceptible, while natural resistance to infection is variable. Recovery from an attack is followed by a temporary immunity which may furnish some protection for years. The organism is easily killed by drying. It is not viable in pure water, but will survive up to 24 hours in sewage, and as long as 6 weeks in certain types of relatively impure water containing organic matter. It can withstand freezing for 3 to 4 days. It is readily killed by dry heat at 117 ° C, by steam and boiling, by short exposure to ordinary disinfectants, and by chlorination of water.

HISTORY AND SIGNIFICANCE

This agent has purportedly been investigated in the past as a biological weapon. Cholera does not easily spread from person-to-person. Therefore, to be an effective biological weapon, major drinking water supplies would need to be heavily contaminated. Recent naturally occurring cholera epidemics in South America have shown the devastating consequences of this disease. Cholera spread quickly in Peru and neighboring countries, despite all attempts to curb the epidemic at an early stage. Over 250,000 symptomatic cases have been reported in Peru alone, and the epidemic has spread to other countries. The rate of symptomatic to asymptomatic cases is 1:400, a factor mitigating against effective use of cholera as a BW agent.

CLINICAL FEATURES

Cholera is an acute infectious disease, characterized by sudden onset with nausea, vomiting, profuse watery diarrhea with 'rice water' appearance, the rapid loss of body fluids, toxemia, and frequent collapse. Mortality can range as high as 50 percent in untreated cases.

DIAGNOSIS

After an incubation period varying from 4 hours to 5 days (average 2-3 days), presumably dependent upon the dose of ingested organisms, onset is usually rather sudden, although the clinical manifestations range from an asymptomatic carrier state to severe illness. Initially the disease presents with intestinal cramping and painless diarrhea. Vomiting, malaise and headache often accompany the diarrhea, especially

early in the illness. If fever is present, it is usually low grade. Diarrhea may be mild or profuse and watery, with fluid losses exceeding 5 to 10 liters or more per day. Electrolyte loss can explain almost all clinical signs and symptoms. Without treatment, death may result from severe dehydration, hypovolemia and shock.

On microscopic examination of stool samples there are few or no red cells or white cells and almost no protein. The absence of inflammatory cells and erythrocytes reflects the non-invasive character of *V. cholerae* infection of the intestinal lumen. The organism can be identified in liquid stool or enrichment broths by darkfield or phase contrast microscopy, and by identifying darting motile vibrio. The organism must be transported using Cary-Blair medium and then streaked for isolation onto TCBS (Thiosulfate Citrate Bile Salt Sucrose) medium. Bacteriologic identification is not necessary to treat cholera, as it can be diagnosed clinically.

MEDICAL MANAGEMENT

Treatment of cholera depends primarily on replacement of fluid and electrolyte losses. This is best accomplished using oral rehydration therapy with the World Health Organization solution (3.5 g NaCl, 2.5 g $NaHCO_3$, 1.5 g KCl and 20 g of glucose per liter). Intravenous fluid replacement is occasionally needed in patients with persistent vomiting or high rates of stool loss (>10ml/kg/hr). Antibiotics will shorten the duration of diarrhea and thereby reduce fluid losses. Tetracycline (500 mg every 6 hours for 3 days) or doxycycline (300 mg once or 100 mg every 12 hours for 3 days) is generally adequate. However, due to widespread tetracycline resistance, ciprofloxacin (500 mg every 12 hours for 3 days) or erythromycin (500 mg every 6 hours for 3 days) should be considered. For pediatric treatment, tetracycline (50 mg/kg/d divided into 4 doses x 3 days) can be used, as dental staining has only occurred after > 6 courses of treatment lasting 6 or more days. Alternates are erythromycin (40 mg/kg/d divided into 4 doses x 3 days), trimethoprim 8 mg and sulfamethoxazole 40 mg/kg day divided into 2 doses x 3 days, and furazolidone (5 mg/kg/d divided into 4 doses x 3 days or 7 mg/kg x one dose).

PROPHYLAXIS

Vaccine: A licensed, killed vaccine is available for use in those considered to be at risk of exposure, however, it provides only about 50 percent protection that lasts for no more than 6 months. The vaccination schedule is an initial dose followed by a second dose 4 weeks later, with booster doses every 6 months. An inactivated oral vaccine (WC/rBS), which is licensed in Europe, is safe and provides rapid short-term protection. Licensure in the US is anticipated. WC/rBS requires 2 doses and has approximately 85% efficacy lasting 2-3 years for both El Tor and classical biotypes. Live attenuated oral vaccines show much promise, and one, CVD 103-HgR (classical biotype), will probably be available by 1999. There are no O139 serogroup vaccines close to licensure, and none of the above mentioned vaccines provide cross-protection against O139. Primary infection with *V. cholerae* O1 serogroup also provides no immunity against O139.

Prevention: Since the major biological threat from this organism appears to be sabotage of food and water supplies, it would seem justified to state that optimal prophylaxis in these circumstances would not be of a medical nature but would be proper safeguarding of these supplies to prevent the sabotage. The best way to prevent cholera in an endemic environment is to avoid contaminated water, ice, fruits, vegetables and also raw or undercooked seafood. Personal contact rarely causes infection because of the high inoculum required for infection; however, enteric precautions and careful hand-washing should be employed. Bactericidal solutions (hypochlorite) would provide adequate decontamination.

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GLANDERS

SUMMARY

Signs and Symptoms: Incubation period ranges from 10-14 days after inhalation. Inhalational exposure produces fever, rigors, sweats, myalgia, headache, pleuritic chest pain, cervical adenopathy, splenomegaly, and generalized papular/pustular eruptions. Almost always fatal without treatment.

Diagnosis: Methylene blue stain of exudates may reveal scant small bacilli. CXR may show miliary lesions, small multiple lung abscesses, or bronchopneumonia. *B. mallei* can be cultured from infected secretions using meat nutrients.

Treatment: Few antibiotics have been evaluated *in vivo*. Sulfadiazine may be effective in some cases. Ciprofloxacin, doxycycline, and rifampin have *in vitro* efficacy. Extrapolating from melioidosis guidelines, a combination of TMP-SMX + ceftazidime ± gentamicin might be considered.

Prophylaxis: No human or veterinary vaccine. Post-exposure prophylaxis may be tried with TMP-SMX.

Isolation and Decontamination: Standard Precautions for healthcare workers. Person-to-person airborne transmission is unlikely, although secondary cases may occur through improper handling of infected secretions. Environmental decontamination using a 0.5% hypochlorite solution is effective.

OVERVIEW

The causative agent of Glanders is *Burkholderia* (formerly Pseudomonas) *mallei*, a gram-negative bacillus primarily noted for producing disease in horses, mules, and donkeys. In the past man has seldom been infected, despite frequent and often close

contact with infected animals. This may be due to exposure to low concentrations of organisms from infected sites in sick animals and the fact that strains virulent for equids are often less virulent for man. There are four basic forms of disease in horses and man. The acute forms are more common in mules and donkeys and death typically follows in 3 to 4 weeks. The chronic form of the disease is more common in horses and causes generalized lymphadenopathy, multiple skin nodules that ulcerate and drain, and induration, enlargement and nodularity of regional lymphatics on the extremities and in other areas. The lymphatic thickening and induration has been called farcy. Human cases have occurred primarily in veterinarians, horse and donkey caretakers, and abattoir workers. The organism spreads to man by invading the nasal, oral, and conjunctival mucous membranes, by inhalation into the lungs, and by invading abraded or lacerated skin. Aerosols from cultures have been observed to be highly infectious to laboratory workers. Work with this organism in the laboratory requires biosafety level 3 containment practices. Despite the rarity of contagion to man from infected horses and donkeys, the attack rates caused by laboratory aerosols have been as high as 46% and cases have been severe. Since aerosol spread is efficient, and there is no available vaccine or really dependable therapy, *B. mallei* has been viewed as a potential BW agent. The disease in Equidae in its natural form poses a minimal threat to military personnel.

HISTORY AND SIGNIFICANCE

Despite the efficiency of spread in a laboratory setting, glanders has only been a sporadic disease in man, and no epidemics of human disease have been reported. There have been no naturally acquired cases of human glanders in the United States in over 59 years. Sporadic cases continue to occur in Asia, Africa, the Middle East and South America. During World War I glanders was believed to have been spread deliberately by agents of the Central Powers to infect large numbers of Russian horses and mules on the Eastern Front. This had an effect on troop and supply convoys as well as on artillery movement which were dependent on horses and mules. Human cases in Russia increased with the infections during and after WWI. The Japanese deliberately infected horses, civilians, and prisoners of war with *B. mallei* at the Pinfang (China) Institute during World War II. The United States studied this agent as a possible BW weapon in 1943-44 but did not weaponize it. The former Soviet Union is believed to have been interested in *B. Mallei* as a potential BW agent after World War II. The low transmission rates of *B. mallei* to man from infected horses is exemplified by the fact that in China, during World War II, thirty percent of tested horses were positive for glanders, but human cases were rare. In Mongolia, 5-25% of tested animals were reactive to *B. mallei*, but no human cases were seen. *B. mallei* exists in nature only in infected susceptible hosts and is not found in water, soil, or plants.

CLINICAL FEATURES

Glanders may occur in an acute localized form, as a septicemic rapidly fatal illness, or as an acute pulmonary infection. Combinations of these syndromes commonly occur in

human cases. A chronic cutaneous form with lymphangitis and regional adenopathy is also frequent.

Aerosol infection produced by a BW weapon containing *B. mallei* could produce any of these syndromes. The incubation period ranges from 10- 14 days, depending on the inhaled dose and agent virulence. The septicemic form begins suddenly with fever, rigors, sweats, myalgia, pleuritic chest pain, photophobia, lacrimation, and diarrhea. Physical examination may reveal fever, tachycardia, cervical adenopathy and mild splenomegaly. Blood cultures are usually negative until the patient is moribund. Mild leukocytosis with a shift to the left or leukopenia may occur.

The pulmonary form may follow inhalation or arise by hematogenous spread. Systemic symptoms as described for the septicemic form occur. Chest radiographs may show miliary nodules (0.5-1.0 cm) and/or a bilateral bronchopneumonia, segmental, or lobar pneumonia and necrotizing nodular lesions.

Acute infection of the oral, nasal and/ or conjunctival mucosa can cause mucopurulent, blood streaked discharge from the nose, associated with septal and turbinate nodules and ulcerations. If systemic invasion occurs from mucosal or cutaneous lesions then a papular and/ or pustular rash may occur that can be mistaken for smallpox (another possible BW agent).

The chronic form is unlikely to be present within 14 days after a BW aerosol attack. It is characterized by cutaneous and intramuscular abscesses on the legs and arms. These lesions are associated with enlargement and induration of the regional lymph channels and nodes. Rare cases develop osteomyelitis, brain abscess, and meningitis. Recovery from chronic glanders may occur or the disease may erupt into an acute septicemic illness. Nasal discharge and ulceration are present in 50% of chronic cases.

Diagnosis

Gram stain of lesion exudates reveals small gram negative bacteria. These stain irregularly with methylene blue. *B. mallei* grows slowly on ordinary nutrient agar, but growth is accelerated with addition of 1-5% glucose and or 5% glycerol. Primary isolation requires 48 hours at 37.5 ºC. Growth is also rapid on most meat infusion nutrient media. Agglutination tests are not positive for 7-10 days, and a high background titer in normal sera (1:320 to 1:640) makes interpretation difficult. Complement fixation tests are more specific and are considered positive if the titer is equal to, or exceeds 1:20. Cultures of autopsy nodules in septicemic cases will usually establish the presence of *B. mallei*. Occurrence in the absence of animal contact and/ or in a human epidemic form is presumptive evidence of a BW attack. Mortality will be high despite antibiotic use. In the hamster 1 to 10 organisms administered by aerosol is lethal. "Resistant species" such as albino mouse can be infected with higher inhalation doses.

MEDICAL MANAGEMENT

Standard Precautions should be used to prevent person-to-person transmission in proven or suspected cases. Sulfadiazine 100 mg/kg per day in divided doses for 3 weeks has been found to be effective in experimental animals and in humans. Other antibiotics that have been effective in experimental infection in hamsters include doxycycline, rifampin, trimethoprim-sulfamethoxazole, and ciprofloxacin. The limited number of infections in humans has precluded therapeutic evaluation of most of the antibiotic agents, therefore, most antibiotic sensitivities are based on animal *in vitro* studies. Various isolates have markedly different antibiotic sensitivities, so that each isolate should be tested for its own individual resistance pattern.

PROPHYLAXIS

Vaccine: There is no vaccine available for human use.

Antibiotics: Post-exposure chemoprophylaxis may be tried with TMP-SMX.

PLAGUE

SUMMARY

Signs and Symptoms: Pneumonic plague incubates 2-3 days. High fever, chills, headache, hemoptysis, and toxemia, progressing rapidly to dyspnea, stridor, and cyanosis. Death from respiratory failure, circulatory collapse, and a bleeding diathesis. Bubonic plague incubates 2-10 days. Malaise, high fever, and tender lymph nodes (buboes); may progress spontaneously to the septicemic form, with spread to the CNS, lungs, etc.

Diagnosis: Presumptive diagnosis can be made by Gram or Wayson stain of lymph node aspirates, sputum, or CSF. Plague bacilli may also be cultured on standard media.

Treatment: Early administration of antibiotics is very effective. Supportive therapy is required.

Prophylaxis: A licensed, killed vaccine is available. Primary series of an initial dose followed by a second smaller dose 1-3 months later, and a third dose 5-6 months after the second dose. Give 3 booster doses at 6 month intervals following dose 3 of the primary series then every 1-2 years. This vaccine is effective against bubonic plague, but probably not against aerosol exposure.

Isolation and Decontamination: Standard Precautions for healthcare workers exposed to bubonic plague. Droplet Precautions for healthcare workers exposed to pneumonic plague. Heat, disinfectants (2-5% hypochlorite) and exposure to sunlight renders bacteria harmless.

OVERVIEW

Yersinia pestis, a rod-shaped, non-motile, non-sporulating, gram-negative, bipolar staining, facultative anaerobic bacterium. It causes plague, normally a zoonotic disease of rodents (e.g., rats, mice, ground squirrels). Fleas which live on the rodents can sometimes pass the bacteria to human beings, who then suffer from the bubonic form of plague. The pneumonic form of the disease would be seen as the primary form after purposeful aerosol dissemination of the organisms. The bubonic form would be seen after purposeful dissemination through the release of infected fleas. All human populations are susceptible. Recovery from the disease may be followed by temporary immunity. The organism will probably remain viable in water and moist meals and grains for several weeks. At near freezing temperatures, it will remain alive from months to years but is killed by 15 minutes exposure to 72 ° C. It also remains viable for some time in dry sputum, flea feces, and buried bodies but is killed within several hours of exposure to sunlight.

HISTORY AND SIGNIFICANCE

The United States worked with *Y. pestis* as a potential biowarfare agent in the 1950's and 1960's before the old offensive biowarfare program was terminated, and other countries are suspected of weaponizing this organism. During World War II, there is reported evidence that Japan investigated the use of *Y. pestis* as a biological weapon. It was reported that they worked on a plan for attacking enemy troops with the organism by releasing plague-infected fleas. This bacterium could be delivered theoretically as an aerosol.

CLINICAL FEATURES

Plague normally appears in three forms in man; bubonic, primary septicemic, and pneumonic. The buboes in the bubonic form are normally seen in the inguinal lymph nodes as the legs are the most commonly "flea-bitten" part of the human body. Septicemia is common, as greater than 80 percent of blood cultures are positive for the organism in bubonic plague, although primary septicemia may occur without lymphadenopathy. The pneumonic form is an infection of the lungs due either to inhalation of the organisms (primary pneumonic plague), or spread to the lungs from septicemia (secondary pneumonic plague). In man, the mortality of untreated bubonic plague is approximately 50 percent, whereas in pneumonic plague the mortality rate is 100 percent.

DIAGNOSIS

After an incubation period varying from 2-3 days for primary pneumonic plague, onset is acute and often fulminant. The presentation is one of malaise, high fever, chills, headache, myalgia, cough with production of a bloody sputum, and toxemia. The chest X-ray reveals a patchy or consolidated bronchopneumonia. The pneumonia progresses rapidly, resulting in dyspnea, stridor, and cyanosis. The terminal event is one of respiratory failure, circulatory collapse, and a bleeding diathesis. In bubonic plague the incubation period ranges from 2 to 10 days with the onset also being acute and often

fulminant. The presentation is one of malaise, high fever, and one or more tender lymph nodes. The liver and spleen are often tender and palpable. One quarter of patients will have various types of skin lesions. Occasionally a pustule, vesicle, eschar or papule containing leukocytes and bacteria will be apparent in the bubo distribution and presumably represents the site of the inoculating flea bite. Bubonic plague may progress spontaneously to the septicemic form with organisms spreading to the central nervous system, lungs, and elsewhere. Black necrotic and purpuric lesions caused by endotoxemia are also often present.

Laboratory findings include a leukocytosis, with a total WBC count up to 20,000 cells with increased bands, and greater than 80 percent polymorphonuclear cells. One also often finds increased fibrin split products in the blood indicative of a low-grade DIC, and the ALT, AST, and bilirubin are also elevated.

A presumptive diagnosis can be made microscopically by identification of the gram-negative coccobacillus with safety-pin bipolar staining in Gram or Wayson's stained smears from a lymph node needle aspirate, sputum, or cerebrospinal fluid sample. When available, immunofluorescent staining is very useful. A definitive diagnosis can be readily made by culturing the organism from blood, sputum, and bubo aspirates. The organism grows slowly at normal incubation temperatures, and may be misidentified by automated systems because of delayed biochemical reactions. It may be cultured on blood agar, MacConkey agar or infusion broth. Most naturally occurring strains of *Y. pestis* produce an F1-antigen *in vivo*, which can be detected in serum samples by immunoassay. A four-fold rise in antibody titer in patient serum is also diagnostic.

MEDICAL MANAGEMENT

Use Standard Precautions for healthcare workers exposed to bubonic plague and Droplet Precautions for healthcare workers exposed to pneumonic plague until the patient has been on antibiotic therapy for at least 48 hours and there has been a favorable clinical response to treatment. Streptomycin, tetracycline, chloramphenicol, and gentamicin are highly effective, especially if begun early (within 24 hours of onset of symptoms). Plague pneumonia is almost always fatal if treatment is not initiated within 24 hours of the onset of symptoms. Streptomycin remains the drug of choice and is given 30 mg/kg/day (IM) in two divided doses for ten days. Gentamicin is acceptable if streptomycin is unavailable. While the patient is typically afebrile after 3 days, the extra week of therapy prevents relapses. Intravenous doxycycline 200 mg initially, followed by 100 mg every 12 hours for 10-14 days is also effective. Results obtained from laboratory animal, but not human, experience, indicate that quinolone antibiotics, such as ofloxacin and ciprofloxacin, may also be effective. The addition of chloramphenicol (1 gm IV QID x 10-14 days) is required for the treatment of plague meningitis.

Usual supportive therapy required includes IV crystalloids and hemodynamic monitoring. Although low-grade DIC may occur, clinically significant hemorrhage is uncommon as is the need to treat with heparin. Finally, buboes rarely require incision and drainage or any form of local care, but instead recede with systemic antibiotic

therapy. In fact, incision and drainage may pose a risk to others in contact with the patient.

PROPHYLAXIS

Vaccine: A licensed, killed whole cell vaccine is available for use in those considered to be at risk of exposure. The primary series consists of three doses. The initial dose of 1.0 ml IM followed by 0.2 ml IM at 1 and 6 months. Three booster doses of 0.2 ml IM are given at 6 month intervals following the third dose of the primary series and then every 1-2 years thereafter. The current vaccine offers protection against bubonic plague, but is probably not effective against aerosolized *Y. pestis*. Presently, 8-10 percent of inoculations result in local reactions which include erythema, induration, tenderness and edema at the site of injection. These typically resolve within 48 hours. Approximately 7-10 percent of inoculations will result in systemic symptoms including malaise, lymphadenopathy, fever and very rarely anaphylaxis, tachycardia, urticaria, or hypotension.

Antibiotics: Because of oral administration and relative lack of toxicity, the choice of antibiotic for prophylaxis or for use in face-to-face contacts of patients with pneumonic plague or after a confirmed or suspected plague BW attack is doxycycline 100 mg orally twice daily, for seven days or the duration of risk of exposure, whichever is longer. Ciprofloxacin has also shown to be effective in preventing disease in exposed mice, and may be more available in a wartime setting as it is also distributed in blister-packs for anthrax post-exposure prophylaxis.

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TULAREMIA

SUMMARY

Signs and Symptoms: Ulceroglandular tularemia presents with a local ulcer and regional lymphadenopathy, fever, chills, headache and malaise. Typhoidal tularemia presents with fever, headache, malaise, substernal discomfort, prostration, weight loss and a non-productive cough.

Diagnosis: Clinical diagnosis. Physical findings are usually non-specific. Chest x-ray may reveal a pneumonic process, mediastinal lymphadenopathy or pleural effusion. Routine culture is possible but difficult. The diagnosis can be established retrospectively by serology.

Treatment: Administration of antibiotics (streptomycin or gentamicin) with early treatment is very effective.

Prophylaxis: A live, attenuated vaccine is available as an investigational new drug. It is administered once by scarification. A two week course of tetracycline is effective as prophylaxis when given after exposure.

Isolation and Decontamination: Standard Precautions for healthcare workers. Organisms are relatively easy to render harmless by mild heat (55 degrees Celsius for 10 minutes) and standard disinfectants.

OVERVIEW

Francisella tularensis, the causative agent of tularemia, is a small, aerobic non-motile, gram-negative cocco-bacillus. Tularemia (also known as rabbit fever and deer fly fever) is a zoonotic disease which humans typically acquire after contact of their skin or mucous membranes with tissues or body fluids of infected animals, or from bites of infected deerflies, mosquitoes, or ticks. Less commonly, inhalation of contaminated dusts or ingestion of contaminated foods or water may produce clinical disease. Respiratory exposure by aerosol would cause typhoidal or pneumonic tularemia. *F. tularensis* can remain viable for weeks in water, soil, carcasses, and hides, and for years in frozen rabbit meat. It is resistant for months to temperatures of freezing and below. It is rather easily killed by heat and disinfectants.

HISTORY AND SIGNIFICANCE

Tularemia was recognized in Japan in the early 1800's and in Russia in 1926. In the early 1900's, American workers investigating suspected plague epidemics in San Francisco isolated the organism and named it *Bacterium tularense* after Tulare County where the work was performed. Dr. Edward Francis, USPHS, established the cause of deer-fly fever as *Bacterium tularense* and subsequently devoted his life to researching the organism and disease, hence, the organism was later renamed *Francisella tularensis*

Francisella tularensis was weaponized by the United States in the 1950's and 1960's during the U.S. offensive biowarfare program, and other countries are suspected to have weaponized this agent. This organism could potentially be stabilized for weaponization by an adversary and theoretically produced in either a wet or dried form. It could then theoretically be delivered against U.S. forces in a similar fashion to the other bacteria discussed in this handbook.

CLINICAL FEATURES

After an incubation period varying from 1-21 days (average 3-5 days), presumably dependent upon the dose of organisms, onset is usually acute. Tularemia may appear in several forms in man depending upon the route of inoculation: ulceroglandular, glandular, typhoidal, oculoglandular, pharyngeal, and pneumonic tularemia. In humans, as few as 10 to 50 organisms will cause disease if inhaled or injected intradermally, whereas approximately 10^8 organisms are required with oral challenge.

Ulceroglandular tularemia (75-85 percent of cases) is most often acquired through inoculation of the skin or mucous membranes with blood or tissue fluids of infected animals. It is characterized by fever, chills, headache, and malaise, an ulcerated skin lesion and painful regional lymphadenopathy. The skin lesion is usually located on the fingers or hand.

Glandular tularemia (5-10 percent of cases) results in fever and tender lymphadenopathy but no skin ulcer.

Typhoidal tularemia accounts for 5-15 percent of naturally occurring cases and occurs mainly after inhalation of infectious aerosols, but can occur after intradermal or gastrointestinal challenge. It manifests as fever, prostration, and weight loss but without lymphadenopathy. Pneumonia may be associated with any form but is most common in typhoidal tularemia. Diagnosis of primary typhoidal tularemia is difficult, as signs and symptoms are non-specific and there frequently is no suggestive exposure history. Respiratory symptoms, substernal discomfort, and a non-productive cough may also be present. Radiologic evidence of pneumonia or mediastinal lymphadenopathy is most common with typhoidal disease but may or may not be present in all other forms of tularemia.

Oculoglandular tularemia (1-2 percent of cases) occurs after inoculation of the conjunctivae with infectious material. Patients have unilateral, painful, purulent conjunctivitis with preauricular or cervical lymphadenopathy. Chemosis, periorbital edema, and small nodular lesions or ulcerations of the palpebral conjunctiva are noted in some patients.

Oropharyngeal tularemia refers to primary ulceroglandular disease confined to the throat. It produces an acute exudative or membranous pharyngotonsillitis with cervical lymphadenopathy.

Pneumonic tularemia is an illness characterized primarily by pneumonia. Pneumonia is common in tularemia. It is seen in 30-80 percent of the typhoidal cases and in 10-15 percent of the ulceroglandular cases. The case fatality rate without treatment is approximately 5 percent for the ulceroglandular form and 35 percent for the typhoidal form. All ages are susceptible, and recovery is generally followed by permanent immunity.

DIAGNOSIS

Identification of organisms by staining ulcer fluids or sputum is generally not helpful. Routine culture is difficult, due to unusual growth requirements and/or overgrowth of commensal bacteria. Isolation represents a clear hazard to laboratory personnel and should only be attempted in BL-3 laboratory. The diagnosis can be established retrospectively serologically. A fourfold rise in the tularemia tube agglutination or microagglutination titer is diagnostic of infection. A single convalescent titer of 1:160 or greater is diagnostic of past or current infection. Titers are usually negative the first week of infection, positive the second week in 50-70 percent of cases and reach a maximum in 4-8 weeks.

MEDICAL MANAGEMENT

Standard Precautions are recommended for healthcare workers. Streptomycin (1 gm every 12 hours IM for 10-14 days) is the treatment of choice. Gentamicin 3-5 mg/kg/day divided TID parenterally for 10-14 days is also effective. Tetracycline and chloramphenicol treatment are effective as well, but are associated with significant relapse rates. Although laboratory related infections with this organism are very common, person-to-person spread is unusual and respiratory isolation is not required.

PROPHYLAXIS

Vaccine: A live, attenuated tularemia vaccine is available as an investigational new drug (IND). It is given by scarification. This vaccine has been administered to more than 5,000 persons without significant adverse reactions. This live vaccine strain (LVS) is of proven effectiveness in preventing laboratory acquired tularemia as well as in aerosol challenged human volunteers. LVS prevents typhoidal and ameliorates the ulceroglandular form of tularemia. As with all vaccines, the degree of protection depends upon the magnitude of the challenge dose; vaccine-induced protection could be overwhelmed by extremely high doses.

Antibiotics: Tetracycline 500 mg PO qid for two weeks is effective as prophylaxis when given after exposure.

Q FEVER

SUMMARY

Signs and Symptoms: Fever, cough, and pleuritic chest pain may occur as early as ten days after exposure. Patients are not generally critically ill, and the illness lasts from 2 days to 2 weeks.

Diagnosis: Q fever is not a clinically distinct illness and may resemble a viral illness or other types of atypical pneumonia. The diagnosis is confirmed serologically.

Treatment: Q fever is generally a self-limited illness even without treatment. Tetracycline or doxycycline are the treatments of choice and are given orally for 5 to 7 days. Q fever endocarditis (rare) is much more difficult to treat.

Prophylaxis: Treatment with tetracycline during the incubation period may delay but not prevent the onset of symptoms. An inactivated whole cell IND vaccine is effective in eliciting protection against exposure, but severe local reactions to this vaccine may be seen in those who already possess immunity.

Isolation and Decontamination: Standard Precautions are recommended for healthcare workers. Person-to-person transmission is rare. Patients exposed to Q fever by aerosol do not present a risk for secondary contamination or re-aerosolization of the organism. Decontamination is accomplished with soap and water or after a 30 minute contact time with 5% microchem plus (quaternary ammonium compound) or 70% ethyl alcohol.

OVERVIEW

The endemic form of Q fever is a zoonotic disease caused by a rickettsia, *Coxiella burnetii*. Its natural reservoirs are sheep, cattle and goats, and grows to especially high concentrations in placental tissues. Exposure to infected animals at parturition is an important risk factor for endemic disease. The organisms are also excreted in animal milk, urine, and feces. Humans acquire the disease by inhalation of aerosols contaminated with the organisms. Farmers and abattoir workers are at greatest risk occupationally. A biological warfare attack with Q fever would cause a disease similar to that occurring naturally. Q fever is also a significant hazard in laboratory personnel who are working with the organism.

HISTORY AND SIGNIFICANCE

Q fever was first described in Australia: it was called "Query fever" because the causative agent was initially unknown. *Coxiella burnetii*, the causative agent, was discovered in 1937. This organism is a rickettsial agent that is resistant to heat and desiccation and highly infectious by the aerosol route. A single inhaled organism may produce clinical illness. For all of these reasons, Q fever could be used as a biological warfare agent. This organism could be employed by an adversary as an incapacitating agent due to its highly infectious nature and likelihood of causing disease if delivered by the respiratory route.

CLINICAL FEATURES

Following the usual incubation period of 10-40 days, Q fever generally occurs as a self-limiting febrile illness lasting 2 days to 2 weeks. The incubation period varies according

to the numbers of organisms inhaled, with longer periods between exposure and illness with lower numbers of inhaled organisms (up to forty days in some cases). The disease generally presents as an acute nondifferentiated febrile illness, with headaches, fatigue, and myalgias as prominent symptoms. Pneumonia manifested by an abnormal chest X-ray occurs in half of all patients, but only half of these, or 25 percent of patients, will have a cough (usually non-productive) or rales. Pleuritic chest pain occurs in about one-fourth of patients with Q fever pneumonia. Chest radiograph abnormalities, when present, are patchy infiltrates that may resemble viral or mycoplasma pneumonia. Rounded opacities and adenopathy have also been described.

Uncommon complications include chronic hepatitis, culture-negative endocarditis, aseptic meningitis, encephalitis and osteomyelitis. Most patients who develop endocarditis have pre-existing valvular heart disease.

DIAGNOSIS

Routine Laboratory Findings: The white blood cell count is elevated in one third of patients. Most patients with Q fever have a mild elevation of hepatic transaminase levels.

Differential Diagnosis: As Q fever usually presents as an undifferentiated febrile illness, or a primary atypical pneumonia, it may be difficult to distinguish from viral illnesses and must be differentiated from pneumonia caused by *Mycoplasma pneumoniae, Legionella pneumophila, Chlamydia psittaci*, and *Chlamydia pneumoniae* (TWAR). More rapidly progressive forms of Q fever pneumonia may look like bacterial pneumonias such as tularemia or plague. Significant numbers of soldiers (from the same geographic area) presenting over a one to two week period with a nonspecific febrile illness, with associated pneumonic symptoms in about half of cases, should trigger the possibility of an attack with aerosolized Q fever in the minds of the treating physicians. The diagnosis will often rest on the clinical and epidemiologic picture in the setting of a possible biowarfare attack.

Specific Laboratory Diagnosis: Identification of organisms by examination of the sputum is not helpful. Isolation of the organism is impractical, as the organism is difficult to culture and a significant hazard to laboratory workers. Serological tests for Q fever include identification of antibody to *C. burnetii* by indirect fluorescent antibody (IFA), enzyme-linked immunosorbent assay (ELISA), and complement fixation. Specific IgM antibodies may be detectable as early as the second week after onset of illness. ELISA testing is available at USAMRIID. A single serum specimen can be used to reliably diagnose acute Q fever with this test as early as 1 1/2 - 2 weeks into the illness. The most commonly available serologic test is the complement fixation test (CF) which is

relatively insensitive and may not be useful if sera have intrinsic anti-complement activity.

MEDICAL MANAGEMENT

Standard Precautions are recommended for healthcare workers. Most cases of acute Q fever will eventually resolve without antibiotic treatment. Tetracycline 500 mg every 6 hr or doxycycline 100 mg every 12 hr for 5-7 days will shorten the duration of illness, and fever usually disappears within one to two days after treatment is begun. Successful treatment of Q fever endocarditis is much more difficult. Tetracycline or doxycycline given in combination with trimethoprim-sulfamethoxazole (TMP-SMX) or rifampin for 12 months or longer has been successful in some cases. However, valve replacement is often required to achieve a cure.

PROPHYLAXIS

Vaccine: A formalin-inactivated whole cell IND vaccine is available for immunization of at-risk personnel on an investigational basis, although a Q fever vaccine is licensed in Australia. Vaccination with a single dose of this killed suspension of *C. burnetii* provides complete protection against naturally occurring Q fever, and greater than 95 percent protection against aerosol exposure. Protection lasts for at least 5 years. Administration of this vaccine in immune individuals may cause severe local induration, sterile abscess formation, and even necrosis at the inoculation site. This observation led to the development of an intradermal skin test using 0.02 mg of specific formalin-killed whole-cell vaccine to detect presensitized or immune individuals.

Antibiotics: Tetracycline or doxycycline given prophylactically after exposure can delay the onset of disease, or even prevent symptoms if administered late in the incubation period. When prophylaxis is started one day after exposure and continued for 5 days, clinical disease has been shown to occur about three weeks after stopping therapy. If prophylaxis is begun 8 to 12 days post-exposure and continued for 5 days, clinical disease will not occur after treatment is discontinued.

VIRAL AGENTS

Viruses are the simplest type of microorganism and consist of a nucleocapsid protein coat containing genetic material, either RNA or DNA. In some cases the virus particle is also surrounded by an outer layer of lipids. Viruses are much smaller than bacteria and vary in size from 0.02 μ m to 0.2 μ m (1 μ m = 1/1000 mm). Viruses lack a system for

their own metabolism and are therefore dependent on the synthetic machinery of their host cells: viruses are thus intracellular parasites. This also means that the virus, unlike the bacterium, cannot be cultivated in synthetic nutritive solutions but requires living cells in order to multiply. The host cells can be from human beings, animals, plants, or bacteria. Every virus needs its own special type of host cell because a complicated interaction is required between the cell and virus if the virus is to be able to multiply. Many virus-specific host cells can be cultivated in synthetic nutrient solutions and afterwards can be infected with the virus in question. Another usual way of cultivating viruses is to let them grow on chorioallantoic membranes (from fertilized eggs). The cultivation of viruses is costly, demanding, and time-consuming. A virus normally brings about changes in the host cell such that the cell dies. This handbook will cover a virus considered by some to be the most likely viral agent that would be used in a BW attack, the alpha virus that causes Venezuelan equine encephalitis, known as VEE. We also discuss smallpox and hemorrhagic fever viruses which could potentially be employed as BW agents.

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SMALLPOX

SUMMARY

Signs and Symptoms: Clinical manifestations begin acutely with malaise, fever, rigors, vomiting, headache, and backache. 2-3 days later lesions appear which quickly progress from macules to papules, and eventually to pustular vesicles. They are more abundant on the extremities and face, and develop synchronously.

Diagnosis: Electron and light microscopy are not capable of discriminating variola from vaccinia, monkeypox or cowpox. The new PCR diagnostic techniques may be more accurate in discriminating between variola and other *Orthopoxviruses*.

Treatment: At present there is no effective chemotherapy, and treatment of a clinical case remains supportive.

Prophylaxis: Immediate vaccination or revaccination should be undertaken for all personnel exposed. Vaccinia immune globulin (VIG) is of value in post-exposure prophylaxis of smallpox when given within the first week following exposure.

Isolation and Decontamination: Droplet and Airborne Precautions for a minimum of 16-17 days following exposure for *all* contacts. Patients should be considered infectious until all scabs separate.

OVERVIEW

Variola virus causes smallpox. It is an Orthopox virus and occurs in at least two strains, variola major and the milder disease, variola minor. Despite the global eradication of smallpox and continued availability of a vaccine, the potential weaponization of variola continues to pose a military threat. This threat can be attributed to the aerosol infectivity of the virus, the relative ease of large-scale production, and an increasingly *Orthopoxvirus*-naive populace. Although the fully-developed cutaneous eruption of smallpox is unique, earlier stages of the rash could be mistaken for varicella. Secondary spread of infection constitutes a nosocomial hazard from the time of onset of a smallpox patient's exanthem until scabs have separated. Quarantine with respiratory isolation should be applied to secondary contacts for 17 days post-exposure. Vaccinia vaccination and vaccinia immune globulin each possess some efficacy in post-exposure prophylaxis.

HISTORY AND SIGNIFICANCE

Endemic smallpox was declared eradicated in 1980 by the World Health Organization (WHO). Although two WHO-approved repositories of variola virus remain at the Centers for Disease Control and Prevention (CDC) in Atlanta and the Institute for Viral Preparations in Moscow, the extent of clandestine stockpiles in other parts of the world remains unknown. In January 1996, WHO’s governing board recommended that all stocks of smallpox be destroyed by 30 June, 1999.

The United States stopped vaccinating its military population in 1989 and civilians in the early 1980s. These populations are now susceptible to variola major, although recruits immunized in 1989 may retain some degree of immunity. Variola may have been used by the British Army against native Americans by giving them contaminated blankets from the beds of smallpox victims during the eighteenth century. Japan considered the use of smallpox as a BW weapon in World War II and it has been considered as a possible threat agent against US forces for many years.

CLINICAL FEATURES

The incubation period of smallpox averaged 12 days, and contacts were quarantined for a minimum of 16-17 days following exposure. Clinical manifestations began acutely with malaise, fever, rigors, vomiting, headache, and backache; 15% of patients developed delirium. Approximately 10% of light-skinned patients exhibited an erythematous rash during this phase. Two to three days later, an enanthem appeared concomitantly with a discrete rash about the face, hands and forearms.

Following eruptions on the lower extremities, the rash spread centrally to the trunk over the next week. Lesions quickly progressed from macules to papules, and eventually to pustular vesicles. Lesions were more abundant on the extremities and face, and this centrifugal distribution is an important diagnostic feature. In distinct contrast to varicella, lesions on various segments of the body remained generally synchronous in their stage of development. From 8 to 14 days after onset, the pustules formed scabs which leave depressed depigmented scars upon healing. Although variola concentrations in the

throat, conjunctiva, and urine diminished with time, virus could readily be recovered from scabs throughout convalescence. Therefore, patients should be isolated and considered infectious until all scabs separate.

For the past century, two distinct types of smallpox were recognized. Variola minor was distinguished by milder systemic toxicity and more diminutive pox lesions, and caused 1% mortality in unvaccinated victims. However, the prototypical disease variola major caused mortality of 3% and 30% in the vaccinated and unvaccinated, respectively. Other clinical forms associated with variola major, flat-type and hemorrhagic-type smallpox, were notable for severe mortality. A naturally occurring relative of variola, monkeypox, occurs in Africa, and is clinically indistinguishable from smallpox with the exception of notable enlargement of cervical and inguinal lymph nodes.

DIAGNOSIS

Smallpox must be distinguished from other vesicular exanthems, such as chickenpox, erythema multiforme with bullae, or allergic contact dermatitis. Particularly problematic to infection control measures would be the failure to recognize relatively mild cases of smallpox in persons with partial immunity. An additional threat to effective quarantine is the fact that exposed persons may shed virus from the oropharynx without ever manifesting disease. Therefore, quarantine and initiation of medical countermeasures should be promptly followed by an accurate diagnosis so as to avert panic.

The usual method of diagnosis is demonstration of characteristic virions on electron microscopy of vesicular scrapings. Under light microscopy, aggregations of variola virus particles, called Guarnieri bodies, are found. Another rapid but relatively insensitive test for Guarnieri bodies in vesicular scrapings is Gispen's modified silver stain, in which cytoplasmic inclusions appear black.

None of the above laboratory tests are capable of discriminating variola from vaccinia, monkeypox or cowpox. This differentiation classically required isolation of the virus and characterization of its growth on chorioallantoic membrane. The development of polymerase chain reaction diagnostic techniques promises a more accurate and less cumbersome method of discriminating between variola and other *Orthopoxviruses*.

MEDICAL MANAGEMENT

Medical personnel must be prepared to recognize a vesicular exanthem in possible biowarfare theaters as potentially variola, and to initiate appropriate countermeasures. Any confirmed case of smallpox should be considered an international emergency with immediate report made to public health authorities. Droplet and Airborne Precautions for a minimum of 16-17 days following exposure for *all* persons in direct contact with the index case, especially the unvaccinated. Patients should be considered infectious until all scabs separate. Immediate vaccination or revaccination should also be undertaken for all personnel exposed to either weaponized variola virus or a clinical case of smallpox.

The potential for airborne spread to other than close contacts is controversial. In general, close person-to-person proximity is required for transmission to reliably occur. Nevertheless, variola's potential in low relative humidity for airborne dissemination was alarming in two hospital outbreaks. Smallpox patients were infectious from the time of onset of their eruptive exanthem, most commonly from days 3-6 after onset of fever. Infectivity was markedly enhanced if the patient manifested a cough. Indirect transmission via contaminated bedding or other fomites was infrequent. Some close contacts harbored virus in their throats without developing disease, and hence might have served as a means of secondary transmission.

Vaccination with a verified clinical "take" (vesicle with scar formation) within the past 3 years is considered to render a person immune to smallpox. However, given the difficulties and uncertainties under wartime conditions of verifying the adequacy of troops' prior vaccination, routine revaccination of all potentially exposed personnel would seem prudent if there existed a significant prospect of smallpox exposure.

Antivirals for use against smallpox are under investigation. Cidofovir has been shown to have significant *in vitro* and *in vivo* activity in experimental animals.

PROPHYLAXIS

Vaccine: Smallpox vaccine (vaccinia virus) is most often administered by intradermal inoculation with a bifurcated needle, a process that became known as scarification because of the permanent scar that resulted. Vaccination after exposure to weaponized smallpox or a case of smallpox is effective in preventing disease if given within 7 days after exposure. A vesicle typically appears at the vaccination site 5-7 days post-inoculation, with surrounding erythema and induration. The lesion forms a scab and gradually heals over the next 1-2 weeks.

Side effects include low-grade fever and axillary lymphadenopathy. The attendant erythema and induration of the vaccination vesicle is frequently misdiagnosed as bacterial superinfection. More severe first-time vaccine reactions include secondary inoculation of the virus to other sites such as the face, eyelid, or other persons (~ 6/10,000 vaccinations), and generalized vaccinia, which is a systemic spread of the virus to produce mucocutaneous lesions away from the primary vaccination site (~3/10,000 vaccinations).

Vaccination is *contraindicated* in the following conditions: immunosuppression, HIV infection, history or evidence of eczema, or current household, sexual, or other close physical contact with person(s) possessing one of these conditions. In addition, vaccination should not be performed during pregnancy.

Despite the above caveats, most authorities state that, with the exception of significant impairment of systemic immunity, there are no absolute contraindications to *post-exposure* vaccination of a person who experiences *bona fide* exposure to variola.

However, concomitant VIG administration is recommended for pregnant and eczematous persons in such circumstances.

Passive Immunoprophylaxis: Evidence indicates that vaccinia immune globulin is of value in post-exposure prophylaxis of smallpox when given within the first week following exposure, and concurrently with vaccination. Vaccination alone is recommended for those without contraindications to the vaccine, unless greater than one week has elapsed after exposure. At this time, administration of both products, if available, is recommended.

The U.S. Army maintains a supply of VIG. The dose for prophylaxis or treatment is 0.6 ml/kg intramuscularly. VIG should be available when using vaccinia vaccine for treatment of adverse reactions.

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VENEZUELAN EQUINE ENCEPHALITIS

SUMMARY

Signs and Symptoms: Sudden onset of illness with generalized malaise, spiking fevers, rigors, severe headache, photophobia, and myalgias. Nausea, vomiting, cough, sore throat, and diarrhea may follow. Full recovery takes 1-2 weeks.

Diagnosis: Clinical diagnosis. Physical findings are usually non-specific. The white blood cell count often shows a striking leukopenia and lymphopenia. Virus isolation may be made from serum, and in some cases throat swab specimens. Both neutralizing or IgG antibody in paired sera or VEE specific IgM present in a single serum sample indicate recent infection.

Treatment: Supportive only.

Prophylaxis: A live, attenuated vaccine is available as an investigational new drug. A second, formalin-inactivated, killed vaccine is available for boosting antibody titers in those initially receiving the live vaccine.

Isolation and Decontamination: Standard Precautions for healthcare workers. Human cases are infectious for mosquitoes for at least 72 hours. The virus can be destroyed by heat (80 degrees centigrade for 30 minutes) and standard disinfectants.

OVERVIEW

Venezuelan equine encephalitis (VEE) virus is an arthropod-borne alphavirus that is endemic in northern South America, Trinidad, Central America, Mexico, and Florida. Eight serologically distinct viruses belonging to the VEE complex have been associated

with human disease; the two most important of these pathogens are designated subtype I, variants A/B, and C. These agents also cause severe disease in horses, mules, burros and donkeys (Equidae). Natural infections are acquired by the bites of a wide variety of mosquitoes. Equidae serve as amplifying hosts and source of mosquito infection. In natural human epidemics, severe and often fatal encephalitis in Equidae always precedes disease in humans. The virus is rather easily killed by heat and disinfectants.

HISTORY AND SIGNIFICANCE

VEE was weaponized by the United States in the 1950's and 1960's before the U.S. offensive biowarfare program was terminated, and other countries have been or are suspected to have weaponized this agent. This virus could theoretically be produced in either a wet or dried form and potentially stabilized for weaponization. This agent could then theoretically be delivered against friendly forces in a manner similar to the other agents already discussed.

As mentioned above, in natural human epidemics, disease in Equidae always precedes that in humans. A biological warfare attack with virus disseminated as an aerosol would almost certainly cause human disease as a primary event. If Equidae were present, disease in these animals would occur simultaneously with human disease. However, during natural epidemics, illness or death in wild or free ranging Equidae may not be recognized before the onset of human disease, thus a natural epidemic could be confused with a BW event, and data on onset of disease should be considered with caution. A more reliable method for determining the likelihood of a BW event would be the presence of VEE outside of its natural geographic range. Secondary spread by person-to-person contact has not been conclusively shown to occur; however, observations during a recent outbreak in Columbia suggest that it may occur often enough to sustain epidemics in the absence of Equidae. A BW attack in a region populated by Equidae and appropriate mosquito vectors could initiate an epizootic/epidemic.

CLINICAL FEATURES

VEE is characterized by inflammation of the meninges of the brain and of the brain itself, thus accounting for the predominance of CNS symptoms in the small percentage of infections that develop encephalitis. The disease is usually acute, prostrating and of short duration. The case fatality rate is less than 1 percent, although is somewhat higher in the very young or aged. Nearly 100 percent of those infected suffer an overt illness. Recovery from an infection results in excellent short-term and long-term immunity.

DIAGNOSIS

After an incubation period varying from 2-6 days, onset is usually sudden. It is manifested by generalized malaise, spiking fever, rigors, severe headache, photophobia, and myalgias in the legs and lumbosacral area. Nausea, vomiting, cough,

sore throat, and diarrhea may follow. This acute phase lasts 24-72 hours. A prolonged period of asthenia and lethargy may follow, with full health and activity regained after 1-2 weeks. Approximately 4 percent of children during natural epidemics develop signs of central nervous system infection, with meningismus, convulsions, coma, and paralysis. Adults rarely develop neurologic complications. In children manifesting severe encephalitis, the fatality rate may reach 20 percent. Permanent neurologic sequelae are reported in survivors. Experimental aerosol challenges in animals suggest that the incidence of CNS disease and associated morbidity and mortality would be high after a BW attack, as the VEE virus would infect the olfactory nerve and spread directly to the CNS. A VEE infection during pregnancy may cause encephalitis in the fetus, placental damage, abortion, or severe congenital neuroanatomical anomalies.

The white blood cell count shows a striking leukopenia and lymphopenia. In cases with encephalitis, the cerebrospinal fluid may be under increased pressure and contain up to 1,000 white cells/mm^3 (predominantly mononuclear cells) and a mildly elevated protein concentration. Viremia during the acute phase of the illness (but not during encephalitis) is generally high enough to allow detection by antigen-capture enzyme immunoassay. Virus isolation may be made from serum, and in some cases throat swab specimens, by inoculation of cell cultures or suckling mice. A variety of serological tests are applicable, including the IgM ELISA indirect FA, hemagglutination inhibition, complement-fixation, and neutralization. For persons without prior exposure to VEE complex viruses, a presumptive diagnosis may be made by finding IgM antibody in a single serum sample taken 5 to 7 days after onset of illness.

MEDICAL MANAGEMENT

Standard Precautions are recommended for healthcare workers. Person-to-person transmission may *theoretically* occur by means of respiratory droplet infection. There is no specific therapy. Patients with uncomplicated VEE infection may be treated with analgesics to relieve headache and myalgia. Patients who develop encephalitis may require anticonvulsants and intensive supportive care to maintain fluid and electrolyte balance, ensure adequate ventilation, and avoid complicating secondary bacterial infections. Patients should be treated in a screened room or in quarters treated with a residual insecticide for at least 5 days after onset, or until afebrile, as human cases may be infectious for mosquitoes for at least 72 hours. The virus can be destroyed by heat and disinfectants.

PROPHYLAXIS

Vaccine: An investigational vaccine, designated TC-83, is a live, attenuated cell-culture-propagated vaccine which has been used in several thousand persons to prevent laboratory infections. The vaccine is given as a single 0.5 ml subcutaneous dose. Febrile reactions occur in up to 18 percent of persons vaccinated, and may be moderate to severe in 5 percent, with fever, myalgias, headache, and prostration. Approximately 18 percent of vaccinees fail to develop detectable neutralizing antibodies, but it is unknown whether they are susceptible to clinical infection if

challenged. Contraindications for use include an intercurrent viral infection or pregnancy. TC-83 is a licensed vaccine for Equidae.

A second investigational product that has been tested in humans is the C-84 vaccine, prepared by formalin-inactivation of the TC-83 strain. The vaccine is not used for primary immunization, but is currently used to boost nonresponders to TC-83 (0.5 ml subcutaneously at 2-4 week intervals for up to 3 inoculations or until an antibody response is measured), and probably affords complete protection against aerosol infection from homologous strains in these individuals. As with all vaccines, the degree of protection depends upon the magnitude of the challenge dose; vaccine-induced protection could be overwhelmed by extremely high doses.

Antiviral Drugs: In experimental animals, alpha-interferon and the interferon-inducer poly-ICLC have proven highly effective for post-exposure prophylaxis of VEE. There are no clinical data on which to assess efficacy in humans.

VIRAL HEMORRHAGIC FEVERS

SUMMARY

Signs and Symptoms: VHFs are febrile illnesses which can be complicated by easy bleeding, petechiae, hypotension and even shock, flushing of the face and chest, and edema. Constitutional symptoms such as malaise, myalgias, headache, vomiting, and diarrhea may occur in any of the hemorrhagic fevers.

Diagnosis: Definitive diagnosis rests on specific virologic techniques. Significant numbers of military personnel with a hemorrhagic fever syndrome should suggest the diagnosis of a viral hemorrhagic fever.

Treatment: Intensive supportive care may be required. Antiviral therapy with ribavirin may be useful in several of these infections. Convalescent plasma may be effective in Argentine hemorrhagic fever.

Prophylaxis: The only licensed VHF vaccine is yellow fever vaccine. Prophylactic ribavirin may be effective for Lassa fever, Rift Valley fever, CCHF, and possibly HFRS.

Isolation and Decontamination: Contact Precautions for healthcare workers. Decontamination is accomplished with hypochlorite or phenolic disinfectants. Isolation measures and barrier nursing procedures are indicated.

OVERVIEW

The viral hemorrhagic fevers are a diverse group of human illnesses that are due to RNA viruses from several different viral families: the *Filoviridae*, which consists of Ebola and Marburg viruses; the *Arenaviridae*, including Lassa fever, Argentine and Bolivian hemorrhagic fever viruses; the *Bunyaviridae*, including various members from the

Hantavirus genus, Congo-Crimean hemorrhagic fever virus from the Nairovirus genus, and Rift Valley fever from the *Phlebovirus* genus; and *Flaviviridae*, such as Yellow fever virus, Dengue hemorrhagic fever virus, and others. The viruses may be spread in a variety of ways, and for some there is a possibility that humans could be infected through a respiratory portal of entry. Although evidence for weaponization does not exist for many of these viruses, many are included in this handbook because of their *potential* for aerosol dissemination or weaponization, or likelihood for confusion with similar agents which might be weaponized.

HISTORY AND SIGNIFICANCE

Because these viruses are so diverse and occur in different geographic locations endemically, their full history is beyond the scope of this handbook. However, there are some significant events for each of them that may provide insight into their possible importance as biological threat agents.

Ebola virus disease was first recognized in the western equatorial province of the Sudan and the nearby region of Zaire in 1976; a second outbreak occurred in Sudan in 1979, and in 1995 a large outbreak (316 cases) developed in Kikwit, Zaire from a single index case. Subsequent epidemics have occurred in Gabon and the Ivory Coast. A related virus was isolated from a group of infected cynomolgus monkeys imported into the United States from the Philippines in 1989. As of yet, this Ebola Reston strain has not been determined as a cause of human disease. The African strains have caused severe disease and death, and it is not known why this disease only appears infrequently or why the most recent strain appears to be less pathogenic in humans. Marburg disease has been identified on four occasions as causing disease in man: three times in Africa, and once in Germany, where the virus got its name. The first recognized outbreak of Marburg disease involved 31 infected persons in Germany and Yugoslavia who were exposed to African green monkeys, with 7 fatalities. It is unclear how easily these filoviruses can be spread from human to human, but spread definitely occurs by direct contact with infected blood, secretions, organs, or semen. The reservoir in nature for these viruses is unknown.

Argentine hemorrhagic fever (AHF), caused by the Junin virus, was first described in 1955 in corn harvesters. It is spread in nature through contact with infected rodent excreta. From 300 to 600 cases per year occur in areas of the Argentine pampas. Bolivian hemorrhagic fever, caused by the related Machupo virus, was described subsequent to AHF in northeastern Bolivia. These viruses have caused laboratory infections, and airborne transmission via dusts contaminated with rodent excreta may occur. A related African arenavirus, Lassa virus, causes disease which is widely distributed over West Africa.

Congo-Crimean hemorrhagic fever (CCHF) is a tick-borne disease which occurs in the Crimea and in parts of Africa, Europe and Asia. It can also be spread by contact with infected animals or nosocomially in healthcare settings. Rift Valley fever occurs only in Africa, and can occasionally cause explosive disease outbreaks. Hantavirus disease

was described prior to WW II in Manchuria along the Amur River, later among United Nations troops during the Korean conflict, and since that time in Korea, Japan, and China. Hemorrhagic disease due to hantaviruses also occurs in Europe (usually in a milder form) and a non-hemorrhagic Hantavirus Pulmonary Syndrome occurs in the Americas and probably worldwide.

Yellow fever and dengue fever are two mosquito-borne fevers which can cause a hemorrhagic fever syndrome and have great historic importance in the history of military campaigns and military medicine.

All of these viruses (except for dengue virus) are infectious by aerosol or fomites. Since most patients are viremic, there is a potential for nosocomial transmission to patients, medical staff, and particularly laboratory personnel. Hantavirus infections are an exception, as at the time of presentation, viremia is waning and circulating antibody is present.

The age and sex distributions of each disease as it occurs endemically generally reflect the opportunities for zoonotic exposure to the disease reservoir. The way in which the filoviruses are transmitted to humans is not well understood.

CLINICAL FEATURES

The clinical syndrome which these viruses may cause in humans is generally referred to as viral hemorrhagic fever or VHF. Not all infected patients develop VHF; there is both divergence and uncertainty about which host factors and virus strain differences might be responsible for clinically manifesting hemorrhagic disease. For instance, an immunopathogenic mechanism has been identified for dengue hemorrhagic fever, which is seen only in patients previously infected with a heterologous dengue serotype. The target organ in the VHF syndrome is the vascular bed; correspondingly, the dominant clinical features are usually a consequence of microvascular damage and changes in vascular permeability. Common presenting complaints are fever, myalgia, and prostration; clinical examination may reveal only conjunctival injection, mild hypotension, flushing, and petechial hemorrhages. Full-blown VHF typically evolves to shock and generalized mucous membrane hemorrhage and often is accompanied by evidence of neurologic, hematopoietic, or pulmonary involvement. Apart from epidemiologic and intelligence information, some distinctive clinical features may suggest a specific etiologic agent: high AST elevation correlates with severity of illness from Lassa fever, and jaundice is a poor prognostic sign in yellow fever. Hepatic involvement is common among the VHFs, but a clinical picture dominated by jaundice and other evidence of hepatic failure is only seen in some cases of Rift Valley fever, Congo-Crimean HF, Marburg HF, Ebola HF, and yellow fever. Neurologic symptoms and thrombocytopenia are common in Argentine and Bolivian hemorrhagic fever. Kyanasur Forest disease and Omsk hemorrhagic fever are notable for a concomitant pulmonary involvement, and a biphasic illness with subsequent CNS manifestations. With regard to the Bunyaviruses, copious hemorrhage and nosocomial transmission are typical for Congo-Crimean HF, and retinitis is commonly seen in Rift Valley fever. Renal

insufficiency is proportional to cardiovascular compromise, except in hemorrhagic fever with renal syndrome (HFRS) due to hantaviruses, where renal azotemia is an integral part of the disease process. Mortality may be substantial, ranging from 5 to 20 percent or higher in recognized cases. Ebola outbreaks in Africa have been notable for the extreme prostration and toxicity of the victims, as well as frighteningly high case fatality rates ranging from 50 to 90 percent. This particularly virulent virus could conceivably be chosen by an adversary as a biological warfare agent due to its probable aerosol infectivity and high mortality.

DIAGNOSIS

A detailed travel history and a high index of suspicion are essential in making the diagnosis of VHF. Patients with arenaviral or hantaviral infections often recall having seen rodents during the presumed incubation period, but, since the viruses are spread to man by aerosolized excreta or environmental contamination, actual contact with the reservoir is not necessary. Large mosquito populations are common during Rift Valley fever or flaviviral transmission, but a history of mosquito bite is sufficiently common to be of little assistance, whereas tick bites or nosocomial exposure are of some significance in suspecting Congo-Crimean hemorrhagic fever. Large numbers of military personnel presenting with VHF manifestations in the same geographic area over a short time period should lead treating medical care providers to suspect either a natural outbreak if in an endemic setting, or possibly a biowarfare attack, particularly if this type of disease does not occur naturally in the local area where troops are deployed.

VHF should be suspected in any patient presenting with a severe febrile illness and evidence of vascular involvement (subnormal blood pressure, postural hypotension, petechiae, easy bleeding, flushing of face and chest, non-dependent edema) who has traveled to an area where the virus is known to occur, or where intelligence information suggests a biological warfare threat. Signs and symptoms suggesting additional organ system involvement are common (headache, photophobia, pharyngitis, cough, nausea or vomiting, diarrhea, constipation, abdominal pain, hyperesthesia, dizziness, confusion, tremor), but usually do not dominate the picture with the exceptions listed above under "Clinical Features."

For much of the world, the major differential diagnosis is malaria. It must be borne in mind that parasitemia in patients partially immune to malaria does not prove that symptoms are due to malaria. Typhoid fever, rickettsial, and leptospiral diseases are major confounding infections, with nontyphoidal salmonellosis, shigellosis, relapsing fever, fulminant hepatitis, and meningococcemia being some of the other important diagnoses to exclude. Any condition leading to disseminated intravascular coagulation could present in a confusing fashion, as well as diseases such as acute leukemia, lupus erythematosus, idiopathic or thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome.

Because of recent recognition of their worldwide occurrence, additional consideration should be given to infection with hantavirus. Classic HFRS (also referred to as Korean

hemorrhagic fever or epidemic hemorrhagic fever) has a severe course which progresses sequentially from fever through hemorrhage, shock, renal failure, and polyuria. This clinical form of HFRS is widely distributed in China, the Korean peninsula, and the Far Eastern USSR. Severe disease also is found in some Balkan states, including Bosnia/Serbia and Greece. However, the Scandinavian and most European virus strains carried by bank voles usually produce a milder disease (referred to as nephropathia epidemica) with prominent fever, myalgia, abdominal pain, and oliguria, but without shock or severe hemorrhagic manifestations. Hantavirus Pulmonary Syndrome, recently recognized in the Americas and probably worldwide, lacks hemorrhagic manifestations, but nevertheless carries a very high mortality due to its rapidly progressive and severe pulmonary capillary leak which presents as ARDS.

The clinical laboratory can be very helpful. Thrombocytopenia (exception: Lassa) and leukopenia (exception: Lassa, Hantaan, and some severe CCHF cases) are the rule. Proteinuria and/or hematuria are common, and their presence is the rule for Argentine HF, Bolivian HF, and HFRS. A positive tourniquet test has been particularly useful in Dengue hemorrhagic fever, but should be sought in other hemorrhagic fevers as well.

Definitive diagnosis in an individual case rests on specific virologic diagnosis. Most patients have readily detectable viremia at presentation (exception: hantaviral infections). Rapid enzyme immunoassays can detect viral antigens in acute sera from patients with Lassa, Argentine HF, Rift Valley fever, Congo-Crimean HF, yellow fever and specific IgM antibodies in early convalescence. Lassa- and Hantaan-specific IgM often are detectable during the acute illness. Diagnosis by virus cultivation and identification will require 3 to 10 days or longer. With the exception of dengue, specialized microbiologic containment is required for safe handling of these viruses. Appropriate precautions should be observed in collection, handling, shipping, and processing of diagnostic samples. Both the Centers for Disease Control and Prevention (CDC, Atlanta, Georgia) and the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID, Frederick, Maryland) have diagnostic laboratories functioning at the highest (BL-4 or P-4) containment level.

MEDICAL MANAGEMENT

Contact Precautions required for healthcare workers. General principles of supportive care apply to hemodynamic, hematologic, pulmonary, and neurologic manifestations of VHF, regardless of the specific etiologic agent concerned. Patients generally are either moribund or recovering by the second week of illness, but only intensive care will save the most severely ill patients. Health care providers employing vigorous fluid resuscitation of patients with hemodynamic compromise must be mindful of the propensity of some VHF cases (e.g., hantaviral) for pulmonary capillary leak. Pressor agents are frequently required. Invasive hemodynamic monitoring should be used where normal indications warrant, but extra caution should be exercised with regard to sharp objects and their potential for nosocomial transmission of a viral agent (see below). Intramuscular injections, aspirin and other anticoagulant drugs should be avoided. Restlessness, confusion, myalgia, and hyperesthesia should be managed by

conservative measures and judicious use of sedative, pain-relieving, and amnestic medications. Secondary infections may occur as with any patient undergoing intensive care and invasive procedures, such as intravenous lines and indwelling catheters.

The management of clinical bleeding should follow the same principles as for any patient with a systemic coagulopathy, assisted by coagulation studies. DIC has been implicated specifically in Rift Valley fever and Marburg/Ebola infections, but in most VHF the etiology of the coagulopathy is multifactorial (e.g., hepatic damage, consumptive coagulopathy, and primary marrow injury to megakaryocytes). Dengue HF is a notable case where antibody-mediated enhancement of dengue virus infection of monocytes and cytotoxic T-cell responses to these presented viral antigens precipitates vascular injury and permeability, complement activation, and a systemic coagulopathy.

The investigational antiviral drug ribavirin is available via compassionate use protocols for therapy of Lassa fever, hemorrhagic fever with renal syndrome (HFRS), Congo-Crimean hemorrhagic fever, and Rift Valley fever. Separate Phase III efficacy trials have indicated that parenteral ribavirin reduces morbidity in both HFRS and Lassa fever, in addition to lowering mortality in the latter disease. In the human field trial with HFRS, treatment was effective if begun within the first 4 days of fever, and was continued for 7 days total. For Lassa fever patients, a compassionate use protocol utilizing intravenous ribavirin as a treatment is sponsored by the CDC. Dosages used were slightly different, and continued for 10 days total; treatment is most effective if begun within 7 days of onset. The only significant side effect of ribavirin is a modest anemia related to reversible block in erythropoiesis and mild hemolysis. Although ribavirin has demonstrated teratogenicity in animal studies, its use in a pregnant woman with grave illness from one of these VHFs must be weighed against potential benefit. Safety in infants and children has not been established. A similar dose of ribavirin begun within 4 days of disease may be effective in HFRS patients. It is important to note that ribavirin has poor *in vitro* and *in vivo* activity against either the filoviruses (Marburg and Ebola) or the flaviviruses (Dengue, Yellow Fever, Omsk HF and Kyanasur Forest Disease).

Argentine HF responds to therapy with 2 or more units of convalescent plasma containing adequate amounts of neutralizing antibody and given within 8 days of onset.

PROPHYLAXIS

The only established and licensed virus-specific vaccine available for any of the hemorrhagic fever viruses is Yellow Fever vaccine, which is mandatory for travelers to endemic areas of Africa and South America. Argentine hemorrhagic fever (AHF) vaccine is a live, attenuated, investigational vaccine developed at USAMRIID, which has proved efficacious both in an animal model and in a field trial in South America, and seems to protect against Bolivian hemorrhagic fever (BHF) as well. Both inactivated and

live-attenuated Rift Valley fever vaccines are currently under investigation. There is no currently available vaccine for either the filoviruses or for dengue.

Persons with percutaneous or mucocutaneous exposure to blood, body fluids, secretions, or excretions from a patient with suspected VHF should immediately wash the affected skin surface(s) with soap and water. Mucous membranes should be irrigated with copious amounts of water or saline.

Close personal contacts or medical personnel extensively exposed to blood or secretions from VHF patients (particularly Lassa fever, CCHF, and filoviral diseases) should be monitored for fever and other disease manifestations during a time equal to the established incubation period. A DoD compassionate use protocol exists for prophylactic administration of oral ribavirin to high risk contacts (direct exposure to body fluids) of Congo-Crimean HF patients. A similar post-exposure prophylaxis strategy has been suggested for high contacts of Lassa fever patients. Most patients will tolerate this drug dose well, but patients should be under surveillance for breakthrough disease (especially after drug cessation) or adverse drug effects (principally anemia).

ISOLATION AND CONTAINMENT

It should be noted that strict adherence to Contact Precautions has halted secondary transmission in the vast majority of circumstances. With the exception of dengue (virus present, but no secondary infection hazard) and hantaviruses (infectious virus not present in blood or excreta at the time of diagnosis), VHF patients generally have significant quantities of virus in blood and often other secretions. Special caution must be exercised in handling sharps, needles, and other potential sources of parenteral exposure. Clinical laboratory personnel are also at risk for exposure, and should employ a biosafety cabinet (if available) and barrier precautions when handling specimens.

Caution should be exercised in evaluating and treating the patient with a suspected VHF. Over-reaction on the part of health care providers is inappropriate and detrimental to both patient and staff, but it is prudent to provide as rigorous isolation measures as feasible. These should include: isolation of the patient; stringent adherence to barrier nursing practices; mask, gown, glove, and needle precautions; decontamination of the outside of double-bagged specimens proceeding from the patient’s room; autoclaving or liberal application of hypochlorite or phenolic disinfectants to excreta and other contaminated materials; and biosafety cabinet containment of laboratory specimens undergoing analysis.

Experience has shown that Marburg, Ebola, Lassa, and Congo-Crimean HF viruses may be particularly prone to aerosol nosocomial spread. Well-documented secondary infections among contacts and medical personnel who were not parenterally exposed have occurred. Sometimes this occurred when the acute hemorrhagic disease (as seen in CCHF) mimicked a surgical emergency such as a bleeding gastric ulcer, with subsequent exposure and secondary spread among emergency and operating room personnel. Therefore, when a significant suspicion of one of these diseases exists,

additional management measures should include: an anteroom adjoining the patient's isolation room to facilitate putting on and removing protective barriers and storage of supplies; use of a negative pressure room for patient care if available; minimal handling of the body should the patient die, with sealing of the corpse in leak-proof material for prompt burial or cremation.

No carrier state has ever been observed with any VHF, but excretion of virus in urine (e.g., hantaviruses) or semen (e.g., Argentine hemorrhagic fever) may occur in convalescence.

BIOLOGICAL TOXINS

Toxins are defined as any toxic substance of natural origin produced by an animal, plant, or microbe. They are different from chemical agents such as VX, cyanide, or mustard in that they are not man-made. They are non-volatile, are usually not dermally active (mycotoxins are an exception), and tend to be more toxic per weight than many chemical agents. Their lack of volatility also distinguishes them from many of the chemical threat agents, and is very important in that they would not be either a persistent battlefield threat or be likely to produce secondary or person to person exposures. Many of the toxins, such as low molecular weight toxins and some peptides, are quite stable, as where the stability of the larger protein bacterial toxins is more variable. The bacterial toxins, such as botulinum toxins or shiga toxin, tend to be the most toxic in terms of dose required for lethality (Appendix C), whereas the mycotoxins tend to be among the least toxic compounds, thousands of times less toxic than the botulinum toxins. Some toxins are more toxic by the aerosol route than when delivered orally or parenterally (ricin, saxitoxin, and T2 mycotoxins are examples), whereas botulinum toxins have lower toxicity when delivered by the aerosol route than when ingested. However, botulinum is so toxic inherently that this characteristic does not limit its potential as a biological warfare agent. The utility of many toxins as military weapons is potentially limited by their inherent low toxicity (too much toxin would be required), or by the fact that some, such as saxitoxin, can only feasibly be produced in minute quantities. The relationship between aerosol toxicity and the quantity of toxin required to provide an effective open-air exposure is shown in Appendix D. The lower the lethal dose for fifty percent of those exposed (LD50), in micrograms per kilogram, the less agent would be required to cover a large battlefield sized area. The converse is also true, and means that for some agents such as ricin, very large quantities (tons) would be needed for an effective open-air attack.

Where toxins are concerned, incapacitation as well as lethality must be considered. Several toxins cause significant illness at levels much lower than the level required for lethality, and are thus militarily significant in their ability to incapacitate soldiers.

This manual will cover four toxins considered to be among the most likely toxins which could be used against U.S. forces: botulinum toxins, staphylococcal enterotoxin B (SEB), ricin, and T-2 mycotoxins.

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BOTULINUM

SUMMARY

Signs and Symptoms: Ptosis, generalized weakness, dizziness, dry mouth and throat, blurred vision and diplopia, dysarthria, dysphonia, and dysphagia followed by symmetrical descending flaccid paralysis and development of respiratory failure. Symptoms begin as early as 24-36 hours but may take several days after inhalation of toxin.

Diagnosis: Clinical diagnosis. No routine laboratory findings. Biowarfare attack should be suspected if multiple casualties simultaneously present with progressive descending bulbar, muscular, and respiratory weakness.

Treatment: Intubation and ventilatory assistance for respiratory failure. Tracheostomy may be required. Administration of heptavalent botulinum antitoxin (IND product) may prevent or decrease progression to respiratory failure and hasten recovery.

Prophylaxis: Pentavalent toxoid vaccine (types A, B, C, D, and E) is available as an IND product for those at high risk of exposure.

Isolation and Decontamination: Standard Precautions for healthcare workers. Toxin is not dermally active and secondary aerosols are not a hazard from patients. Hypochlorite (0.5% for 10-15 minutes) and/or soap and water.

OVERVIEW

The botulinum toxins are a group of seven related neurotoxins produced by the bacillus *Clostridium botulinum*. These toxins, types A through G, could be delivered by aerosol over concentrations of troops. When inhaled, these toxins produce a clinical picture very similar to foodborne intoxication, although the time to onset of paralytic symptoms may actually be longer than for foodborne cases, and may vary by type and dose of toxin. The clinical syndrome produced by one or more of these toxins is known as "botulism".

HISTORY AND SIGNIFICANCE

Botulinum toxins have caused numerous cases of botulism when ingested in improperly prepared or canned foods. Many deaths have occurred secondary to such incidents. It is feasible to deliver botulinum toxins as a biological weapon, and other countries have

weaponized or are suspected to have weaponized one or more of this group of toxins. Iraq admitted to a United Nations inspection team in August of 1991 that it had done research on the offensive use of botulinum toxins prior to the Persian Gulf War, which occurred in January and February of that year. Further information given in 1995 revealed that Iraq had not only researched the use of this toxin as a weapon, but had filled and deployed over 100 munitions with botulinum toxin.

TOXIN CHARACTERISTICS

Botulinum toxins are proteins of approximately 150 kD molecular weight which can be produced from the anaerobic bacterium *Clostridium botulinum*. As noted above, there are seven distinct but related neurotoxins, A through G, produced by different strains of the clostridial bacillus. All seven types act by similar mechanisms. The toxins produce similar effects when inhaled or ingested, although the time course may vary depending on the route of exposure and the dose received. Although an aerosol attack is by far the most likely scenario for the use of botulinum toxins, theoretically the agent could be used to sabotage food supplies; enemy special forces or terrorists might use this method in certain scenarios to produce foodborne botulism in those so targeted.

MECHANISM OF TOXICITY

The botulinum toxins as a group are among the most toxic compounds known to man. Appendix C shows the comparative lethality of selected toxins and chemical agents in laboratory mice. Botulinum toxin is the most toxic compound per weight of agent, requiring only 0.001 microgram per kilogram of body weight to kill 50 percent of the animals studied. As a group, bacterial toxins such as botulinum tend to be the most lethal of all toxins. Note that botulinum toxin type A is 15,000 times more toxic than VX and 100,000 times more toxic than Sarin, two of the well known organophosphate nerve agents.

Botulinum toxins act by binding to the presynaptic nerve terminal at the neuromuscular junction and at cholinergic autonomic sites. These toxins then act to prevent the release of acetylcholine presynaptically, and thus block neurotransmission. This interruption of neurotransmission causes both bulbar palsies and the skeletal muscle weakness seen in clinical botulism.

Unlike the situation with nerve agent intoxication, where there is too much acetylcholine due to inhibition of acetylcholinesterase, the problem in botulism is lack of the neurotransmitter in the synapse. Thus, pharmacologic measures such as atropine are not indicated in botulism and would likely exacerbate symptoms.

CLINICAL FEATURES

The onset of symptoms of inhalation botulism may vary from 24 to 36 hours, to several days following exposure. Recent primate studies indicate that the signs and symptoms may in fact not appear for several days when a low dose of the toxin is inhaled versus a

shorter time period following ingestion of toxin or inhalation of higher doses. Bulbar palsies are prominent early, with eye symptoms such as blurred vision due to mydriasis, diplopia, ptosis, and photophobia, in addition to other bulbar signs such as dysarthria, dysphonia, and dysphagia. Skeletal muscle paralysis follows, with a symmetrical, descending, and progressive weakness which may culminate abruptly in respiratory failure. Progression from onset of symptoms to respiratory failure has occurred in as little as 24 hours in cases of foodborne botulism.

Physical examination usually reveals an alert and oriented patient without fever. Postural hypotension may be present. Mucous membranes may be dry and crusted and the patient may complain of dry mouth or even sore throat. There may be difficulty with speaking and with swallowing. Gag reflex may be absent. Pupils may be dilated and even fixed. Ptosis and extraocular muscle palsies may also be observed. Variable degrees of skeletal muscle weakness may be observed depending on the degree of progression in an individual patient. Deep tendon reflexes may be present or absent. With severe respiratory muscle paralysis, the patient may become cyanotic or exhibit narcosis from CO_2 retention.

DIAGNOSIS

The occurrence of an epidemic of cases of a descending and progressive bulbar and skeletal paralysis in afebrile patients points to the diagnosis of botulinum intoxication. Foodborne outbreaks tend to occur in small clusters and have never occurred in soldiers on military rations such as MRE’s (Meals, Ready to Eat). Higher numbers of cases in a theater of operations should raise at least the consideration of a biological warfare attack with aerosolized botulinum toxin. Foodborne outbreaks are theoretically possible in troops on normal "A" rations.

Individual cases might be confused clinically with other neuromuscular disorders such as Guillain-Barre syndrome, myasthenia gravis, or tick paralysis. The edrophonium or Tensilon® test may be transiently positive in botulism, so it may not distinguish botulinum intoxication from myasthenia. The cerebrospinal fluid in botulism is normal and the paralysis is generally symmetrical, which distinguishes it from enteroviral myelitis. Mental status changes generally seen in viral encephalitis should not occur with botulinum intoxication.

It may become necessary to distinguish nerve agent and/or atropine poisoning from botulinum intoxication. Nerve agent poisoning produces copious respiratory secretions and miotic pupils, whereas there is if anything a decrease in secretions in botulinum intoxication. Atropine overdose is distinguished from botulism by its central nervous system excitation (hallucinations and delirium) even though the mucous membranes are dry and mydriasis is present. The clinical differences between botulinum intoxication and nerve agent poisoning are depicted in Appendix E.

Laboratory testing is generally not helpful in the diagnosis of botulism. Survivors do not usually develop an antibody response due to the very small amount of toxin necessary

to produce clinical symptoms. Detection of toxin in serum or gastric contents is possible, and mouse neutralization (bioassay) remains the most sensitive test. Other assays include gel hydralization or ELISA. Serum specimens should be drawn from suspected cases and held for testing at such a facility.

MEDICAL MANAGEMENT

Respiratory failure secondary to paralysis of respiratory muscles is the most serious complication and, generally, the cause of death. Reported cases of botulism prior to 1950 had a mortality of 60%. With tracheotomy or endotracheal intubation and ventilatory assistance, fatalities should be less than five percent. Intensive and prolonged nursing care may be required for recovery which may take several weeks or even months.

Antitoxin: In isolated cases of food-borne botulism, circulating toxin is present, perhaps due to continued absorption through the gut wall. Botulinum antitoxin (equine origin) has been used in those circumstances, and is thought to be helpful. Animal experiments show that after aerosol exposure, botulinum antitoxin can be very effective if given before the onset of clinical signs. Administration of antitoxin is reasonable if disease has not progressed to a stable state.

A trivalent equine antitoxin has been available from the Centers for Disease Control and Prevention for cases of foodborne botulism. This product has all the disadvantages of a horse serum product, including the risks of anaphylaxis and serum sickness. A "despeciated" equine heptavalent antitoxin against types A, B, C, D, E, F, and G has been prepared by cleaving the Fc fragments from horse IgG molecules, leaving $F(ab)_2$ fragments. This product is under advanced development, and is currently available under IND status. Its efficacy is inferred from its performance in animal studies. Disadvantages include a reduced, but theoretical risk of serum sickness.

Use of the antitoxin requires skin testing for horse serum sensitivity prior to administration. Skin testing is performed by injecting 0.1 ml of a 1:10 dilution (in sterile physiological saline) of antitoxin intradermally in the patient’s forearm with a 26 or 27 gauge needle. Monitor the injection site and observe the patient for allergic reaction for 20 minutes. The skin test is positive if any of these allergic reactions occur: hyperemic areola at the site of the injection > 0.5 cm; fever or chills; hypotension with decrease of blood pressure > 20 mm Hg for systolic and diastolic pressures; skin rash; respiratory difficulty; nausea or vomiting; generalized itching. Do NOT administer Botulinum $F(ab')_2$ Antitoxin, Heptavalent (equine derived) if the skin test is positive. If no allergic symptoms are observed, the antitoxin is administered intravenously in a normal saline solution, 10 mls over 20 minutes.

With a positive skin test, desensitization is carried out by administering 0.01 - 0.1 ml of antitoxin subcutaneously, doubling the previous dose every 20 minutes until 1.0 - 2.0 ml can be sustained without any marked reaction.

PROPHYLAXIS

Vaccine: A pentavalent toxoid of *Clostridium botulinum* toxin types A, B, C, D, and E is available under an IND status. This product has been administered to several thousand volunteers and occupationally at-risk workers, and induces serum antitoxin levels that correspond to protective levels in experimental animal systems. The currently recommended primary series of 0, 2, and 12 weeks, then a 1 year booster induces protective antibody levels in greater than 90 percent of vaccinees after one year. Adequate antibody levels are transiently induced after three injections, but decline prior to the one year booster.

Contraindications to the vaccine include sensitivities to alum, formaldehyde, and thimerosal, or hypersensitivity to a previous dose. Reactogenicity is mild, with two to four percent of vaccinees reporting erythema, edema, or induration at the local site of injection which peaks at 24 to 48 hours, then dissipates. The frequency of such local reactions increases with each subsequent inoculation; after the second and third doses, seven to ten percent will have local reactions, with higher incidence (up to twenty percent or so) after boosters. Severe local reactions are rare, consisting of more extensive edema or induration. Systemic reactions are reported in up to three percent, consisting of fever, malaise, headache, and myalgia. Incapacitating reactions (local or systemic) are uncommon. The vaccine should be stored at refrigerator temperatures (not frozen).

Three or more vaccine doses at 0, 2, and 12 weeks, then at 1 year if possible, all by deep subcutaneous injection are recommended for selected individuals or groups judged at high risk for exposure to botulinum toxin aerosols. There is no indication at present for use of botulinum antitoxin as a prophylactic modality except under extremely specialized circumstances.

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STAPHYLOCOCCAL ENTEROTOXIN B

SUMMARY

Signs and Symptoms: From 3-12 hours after aerosol exposure, sudden onset of fever, chills, headache, myalgia, and nonproductive cough. Some patients may develop shortness of breath and retrosternal chest pain. Fever may last 2 to 5 days, and cough may persist for up to 4 weeks. Patients may also present with nausea, vomiting, and diarrhea if they swallow toxin. Presumably, higher exposure can lead to septic shock and death.

Diagnosis: Diagnosis is clinical. Patients present with a febrile respiratory syndrome without CXR abnormalities. Large numbers of soldiers presenting with typical symptoms

and signs of SEB pulmonary exposure would suggest an intentional attack with this toxin.

Treatment: Treatment is limited to supportive care. Artificial ventilation might be needed for very severe cases, and attention to fluid management is important.

Prophylaxis: Use of protective mask. There is currently no human vaccine available to prevent SEB intoxication.

Isolation and Decontamination: Standard Precautions for healthcare workers. Hypochlorite (0.5% for 10-15 minutes) and/or soap and water. Destroy any food that may have been contaminated.

OVERVIEW

Staphylococcus aureus produces a number of exotoxins, one of which is Staphylococcal enterotoxin B, or SEB. Such toxins are referred to as exotoxins since they are excreted from the organism; however, they normally exert their effects on the intestines and thereby are called enterotoxins. SEB is one of the pyrogenic toxins that commonly causes food poisoning in humans after the toxin is produced in improperly handled foodstuffs and subsequently ingested. SEB has a very broad spectrum of biological activity. This toxin causes a markedly different clinical syndrome when inhaled than it characteristically produces when ingested. Significant morbidity is produced in individuals who are exposed to SEB by either portal of entry to the body.

HISTORY AND SIGNIFICANCE

SEB has caused countless endemic cases of food poisoning. Often these cases have been clustered, due to common source exposure in a setting such as a church picnic or other community event in which contaminated food is consumed. Although this toxin would not be likely to produce significant mortality on the battlefield, it could render up to 80 percent or more of exposed personnel clinically ill and unable to perform their mission for 1-2 weeks. Therefore, even though SEB is not generally thought of as a lethal agent, it may severely incapacitate soldiers, making it an extremely important toxin to consider.

TOXIN CHARACTERISTICS

Staphylococcal enterotoxins are extracellular products produced by coagulase-positive staphylococci. They are produced in culture media and also in foods when there is overgrowth of the staph organisms. At least five antigenically distinct enterotoxins have been identified, SEB being one of them. These toxins are heat stable. SEB causes symptoms when inhaled at very low doses in humans: a dose of several logs lower than the lethal dose by the inhaled route would be sufficient to incapacitate 50 percent of

those soldiers so exposed. This toxin could also be used (theoretically) in a special forces or terrorist mode to sabotage food or small volume water supplies.

MECHANISM OF TOXICITY

Staphylococcal enterotoxins produce a variety of toxic effects. Inhalation of SEB can induce extensive pathophysiological changes to include widespread systemic damage and even septic shock. Many of the effects of staphylococcal enterotoxins are mediated by interactions with the host's own immune system. The mechanisms of toxicity are complex, but are related to toxin binding directly to the major histocompatibility complex that subsequently stimulates the proliferation of large numbers of T cell lymphocytes. Because these exotoxins are extremely potent activators of T cells, they are commonly referred to as bacterial superantigens. These superantigens stimulate the production and secretion of various cytokines, such as tumor necrosis factor, interferon, interleukin-1 and interleukin-2, from immune system cells. Released cytokines are thought to mediate many of the toxic effects of SEB.

CLINICAL FEATURES

Relevant battlefield exposures to SEB are projected to cause primarily clinical illness and incapacitation. However, higher exposure levels can presumably lead to septic shock and death. Intoxication with SEB begins 3 to 12 hours after inhalation of the toxin. Victims may experience the sudden onset of fever, headache, chills, myalgias, and a nonproductive cough. More severe cases may develop dyspnea and retrosternal chest pain. Nausea, vomiting, and diarrhea will also occur in many patients due to inadvertently swallowed toxin, and fluid losses can be marked. The fever may last up to five days and range from 103 to 106 degrees F, with variable degrees of chills and prostration. The cough may persist up to four weeks, and patients may not be able to return to duty for two weeks.

Physical examination in patients with SEB intoxication is often unremarkable. Conjunctival injection may be present, and postural hypotension may develop due to fluid losses. Chest examination is unremarkable except in the unusual case where pulmonary edema develops. The chest X-ray is also generally normal, but in severe cases increased interstitial markings, atelectasis, and possibly overt pulmonary edema or an ARDS picture may develop.

DIAGNOSIS

As is the case with botulinum toxins, intoxication due to SEB inhalation is a clinical and epidemiologic diagnosis. Because the symptoms of SEB intoxication may be similar to several respiratory pathogens such as influenza, adenovirus, and mycoplasma, the diagnosis may initially be unclear. All of these might present with fever, nonproductive cough, myalgia, and headache. SEB attack would cause cases to present in large numbers over a very short period of time, probably within a single 24 hour period. Naturally occurring pneumonias or influenza would involve patients presenting over a

more prolonged interval of time. Naturally occurring staphylococcal food poisoning cases would not present with pulmonary symptoms. SEB intoxication tends to progress rapidly to a fairly stable clinical state, whereas pulmonary anthrax, tularemia pneumonia, or pneumonic plague would all progress if left untreated. Tularemia and plague, as well as Q fever, would be associated with infiltrates on chest radiographs. Nerve agent intoxication would cause fasciculations and copious secretions, and mustard would cause skin lesions in addition to pulmonary findings; SEB inhalation would not be characterized by these findings. The dyspnea associated with botulinum intoxication is associated with obvious signs of muscular paralysis, bulbar palsies, lack of fever, and a dry pulmonary tree due to cholinergic blockade; respiratory difficulties occur late rather than early as with SEB inhalation.

Laboratory findings are not very helpful in the diagnosis of SEB intoxication. A nonspecific neutrophilic leukocytosis and an elevated erythrocyte sedimentation rate may be seen, but these abnormalities are present in many illnesses. Toxin is very difficult to detect in the serum by the time symptoms occur; however, a serum specimen should be drawn as early as possible after exposure. Data from rabbit studies clearly show that SEB in the serum is transient; however, it accumulates in the urine and can be detected for several hours post exposure. Therefore, urine samples should be obtained and tested for SEB. Because most patients will develop a significant antibody response to the toxin, acute and convalescent serum should be drawn which may be helpful retrospectively in the diagnosis.

MEDICAL MANAGEMENT

Currently, therapy is limited to supportive care. Close attention to oxygenation and hydration are important, and in severe cases with pulmonary edema, ventilation with positive end expiratory pressure and diuretics might be necessary. Acetaminophen for fever, and cough suppressants may make the patient more comfortable. The value of steroids is unknown. Most patients would be expected to do quite well after the initial acute phase of their illness, but most would generally be unfit for duty for one to two weeks.

PROPHYLAXIS

Although there is currently no human vaccine for immunization against SEB intoxication, several vaccine candidates are in development. Preliminary animal studies have been encouraging and a vaccine candidate is nearing transition to advanced development and safety and immunogenicity testing in man. Experimentally, passive immunotherapy can reduce mortality, but only when given within 4-8 hours after inhaling SEB.

RICIN

SUMMARY

Signs and Symptoms: Weakness, fever, cough and pulmonary edema occur 18-24 hours after inhalation exposure, followed by severe respiratory distress and death from hypoxemia in 36-72 hours.

Diagnosis: Signs and symptoms noted above in large numbers of geographically clustered patients could suggest an exposure to aerosolized ricin. The rapid time course to severe symptoms and death would be unusual for infectious agents. Laboratory findings are nonspecific but similar to other pulmonary irritants which cause pulmonary edema. Specific serum ELISA is available. Acute and convalescent sera should be collected.

Treatment: Management is supportive and should include treatment for pulmonary edema. Gastric decontamination measures should be used if ingested.

Prophylaxis: There is currently no vaccine or prophylactic antitoxin available for human use, although immunization appears promising in animal models. Use of the protective mask is currently the best protection against inhalation.

Isolation and Decontamination: Standard Precautions for healthcare workers. Secondary aerosols should generally not be a danger to health care providers. Weak hypochlorite solutions (0.1% sodium hypochlorite) and/or soap and water can decontaminate skin surfaces.

OVERVIEW

Ricin is a potent protein toxin derived from the beans of the castor plant (*Ricinus communis*). Castor beans are ubiquitous worldwide, and the toxin is fairly easily produced. Ricin is therefore a potentially widely available toxin. When inhaled as a small particle aerosol, this toxin may produce pathologic changes within 8 hours and severe respiratory symptoms followed by acute hypoxic respiratory failure in 36-72 hours. When ingested, ricin causes severe gastrointestinal symptoms followed by vascular collapse and death. This toxin may also cause disseminated intravascular coagulation, microcirculatory failure and multiple organ failure if given intravenously in laboratory animals.

HISTORY AND SIGNIFICANCE

Ricin's significance as a potential biological warfare toxin relates in part to its wide availability. Worldwide, one million tons of castor beans are processed annually in the

production of castor oil; the waste mash from this process is five percent ricin by weight. The toxin is also quite stable and extremely toxic by several routes of exposure, including the respiratory route. Ricin is said to have been used in the assassination of Bulgarian exile Georgi Markov in London in 1978. Markov was attacked with a specially engineered weapon disguised as an umbrella which implanted a ricin-containing pellet into his body.

TOXIN CHARACTERISTICS

Ricin is actually made up of two hemagglutinins and two toxins. The toxins, RCL III and RCL IV, are dimers of about 66,000 daltons molecular weight. The toxins are made up of two polypeptide chains, an A chain and a B chain, which are joined by a disulfide bond. Ricin can be produced relatively easily and inexpensively in large quantities in a fairly low technology setting. It is of marginal toxicity in terms of its LED_{50} in comparison to toxins such as botulinum and SEB (incapacitating dose), so an enemy would have to produce it in larger quantities to cover a significant area on the battlefield. This might limit large-scale use of ricin by an adversary. Ricin can be prepared in liquid or crystalline form, or it can be lyophilized to make it a dry powder. It could be disseminated by an enemy as an aerosol, or it could be used as a sabotage, assassination, or terrorist weapon.

MECHANISM OF TOXICITY

Ricin is very toxic to cells. It acts by inhibiting protein synthesis. The B chain binds to cell surface receptors and the toxin-receptor complex is taken into the cell; the A chain has endonuclease activity and extremely low concentrations will inhibit protein synthesis. In rodents, the histopathology of aerosol exposure is characterized by necrotizing airway lesions causing tracheitis, bronchitis, bronchiolitis, and interstitial pneumonia with perivascular and alveolar edema. There is a latent period of 8 hours post-inhalation exposure before histologic lesions are observed in animal models. In rodents, ricin is more toxic by the aerosol route than by other routes of exposure.

There is little toxicity data in humans. The exact cause of morbidity and mortality would be dependent upon the route of exposure. Aerosol exposure in man would be expected to cause acute lung injury, pulmonary edema secondary to increased capillary permeability, and eventual acute hypoxic respiratory failure.

CLINICAL FEATURES

The clinical picture in intoxicated victims would depend on the route of exposure. After aerosol exposure, signs and symptoms would depend on the dose inhaled. Accidental sublethal aerosol exposures which occurred in humans in the 1940’s were characterized by onset of the following symptoms in four to eight hours: fever, chest tightness, cough, dyspnea, nausea, and arthralgias. The onset of profuse sweating some hours later was commonly the sign of termination of most of the symptoms. Although lethal human aerosol exposures have not been described, the severe

pathophysiologic changes seen in the animal respiratory tract, including necrosis and severe alveolar flooding, are probably sufficient to cause death if enough toxin is inhaled. Time to death in experimental animals is dose dependent, occurring 36-72 hours post inhalation exposure. Humans would be expected to develop severe lung inflammation with progressive cough, dyspnea, cyanosis and pulmonary edema.

By other routes of exposure, ricin is not a direct lung irritant; however, intravascular injection can cause minimal pulmonary perivascular edema due to vascular endothelial injury. Ingestion causes gastrointestinal hemorrhage with hepatic, splenic, and renal necrosis. Intramuscular administration causes severe local necrosis of muscle and regional lymph nodes with moderate visceral organ involvement.

DIAGNOSIS

An attack with aerosolized ricin would be, as with many biological warfare agents, primarily diagnosed by the clinical and epidemiological setting. Acute lung injury affecting a large number of cases in a war zone (where a BW attack could occur) should raise suspicion of an attack with a pulmonary irritant such as ricin, although other pulmonary pathogens could present with similar signs and symptoms. Other biological threats, such as SEB, Q fever, tularemia, plague, and some chemical warfare agents like phosgene, need to be included in a differential diagnosis. Ricin intoxication would be expected to progress despite treatment with antibiotics, as opposed to an infectious process. There would be no mediastinitis as seen with inhalation anthrax. SEB would be different in that most patients would not progress to a life-threatening syndrome but would tend to plateau clinically. Phosgene-induced acute lung injury would progress much faster than that caused by ricin.

Additional supportive clinical or diagnostic features after aerosol exposure to ricin may include the following: bilateral infiltrates on chest radiographs, arterial hypoxemia, neutrophilic leukocytosis, and a bronchial aspirate rich in protein compared to plasma which is characteristic of high permeability pulmonary edema. Specific ELISA testing on serum or immunohistochemical techniques for direct tissue analysis may be used where available to confirm the diagnosis. Ricin is an extremely immunogenic toxin, and acute as well as convalescent sera should be obtained from survivors for measurement of antibody response.

MEDICAL MANAGEMENT

Management of ricin-intoxicated patients again depends on the route of exposure. Patients with pulmonary intoxication are managed by appropriate treatment for pulmonary edema and respiratory support as indicated. Gastrointestinal intoxication is best managed by vigorous gastric decontamination with superactivated charcoal, followed by use of cathartics such as magnesium citrate. Volume replacement of GI fluid losses is important. In percutaneous exposures, treatment would be primarily supportive.

PROPHYLAXIS

The protective mask is effective in preventing aerosol exposure. Although a vaccine is not currently available, candidate vaccines are under development which are immunogenic and confer protection against lethal aerosol exposures in animals. Prophylaxis with such a vaccine is the most promising defense against a biological warfare attack with ricin.

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T-2 MYCOTOXINS

SUMMARY

Signs and symptoms: Exposure causes skin pain, pruritus, redness, vesicles, necrosis and sloughing of epidermis. Effects on the airway include nose and throat pain, nasal discharge, itching and sneezing, cough, dyspnea, wheezing, chest pain and hemoptysis. Toxin also produces effects after ingestion or eye contact. Severe poisoning results in prostration, weakness, ataxia, collapse, shock, and death.

Diagnosis: Should be suspected if an aerosol attack occurs in the form of "yellow rain" with droplets of yellow fluid contaminating clothes and the environment. Confirmation requires testing of blood, tissue and environmental samples.

Treatment: There is no specific antidote. Superactivated charcoal should be given orally if the toxin is swallowed.

Prophylaxis: The only defense is to wear a protective mask and clothing during an attack. No specific immunotherapy

or chemotherapy is available for use in the field.

Isolation and Decontamination: Standard Precautions for healthcare workers. Outer clothing should be removed and exposed skin should be decontaminated with soap and water. Eye exposure should be treated with copious saline irrigation. Once decontamination is complete, isolation is not required. Environmental decontamination requires the use of a hypochlorite solution under alkaline conditions such as 1% sodium hypochlorite and 0.1M NAOH with 1 hour contact time.

OVERVIEW

The trichothecene mycotoxins are low molecular weight (250-500 daltons) nonvolatile compounds produced by filamentous fungi (molds) of the genera *Fusarium*, *Myrotecium, Trichoderma, Stachybotrys* and others. The structures of approximately 150 trichothecene derivatives have been described in the literature. These substances are relatively insoluble in water but are highly soluble in ethanol, methanol and propylene glycol. The trichothecenes are extremely stable to heat and ultraviolet light inactivation. Heating to 1500^{o} F for 30 minutes is required for inactivation, while brief exposure to NaOCl destroys toxic activity. The potential for use as a BW toxin was demonstrated to the Russian military shortly after World War II when flour contaminated with species of *Fusarium* was unknowingly baked into bread that was ingested by civilians. Some developed a protracted lethal illness called alimentary toxic aleukia (ATA) characterized by initial symptoms of abdominal pain, diarrhea, vomiting, prostration, and within days fever, chills, myalgias and bone marrow depression with granulocytopenia and secondary sepsis. Survival beyond this point allowed the development of painful pharyngeal/laryngeal ulceration and diffuse bleeding into the skin (petechiae and ecchymoses), melena, bloody diarrhea, hematuria, hematemesis, epistaxis and vaginal bleeding. Pancytopenia, and gastrointestinal ulceration and erosion were secondary to the ability of these toxins to profoundly arrest bone marrow and mucosal protein synthesis and cell cycle progression through DNA replication.

History AND SIGNIFICANCE

Mycotoxins allegedly have been used in aerosol form ("yellow rain") to produce lethal and nonlethal casualties in Laos (1975-81), Kampuchea (1979-81), and Afghanistan (1979-81). It has been estimated that there were more than 6,300 deaths in Laos, 1,000 in Kampuchea, and 3,042 in Afghanistan. The alleged victims were usually unarmed civilians or guerrilla forces. These groups were not protected with masks or chemical protective clothing and had little or no capability of destroying the attacking enemy aircraft. These attacks were alleged to have occurred in remote jungle areas which made confirmation of attacks and recovery of agent extremely difficult. Some investigators have claimed that the "yellow clouds" were, in fact, bee feces produced by swarms of migrating insects. Much controversy has centered upon the veracity of eyewitness and victim accounts, but there is evidence to make these allegations of BW agent use in these areas possible.

CLINICAL FEATURES

T-2 and other mycotoxins may enter the body through the skin and digestive or respiratory epithelium. They are fast acting potent inhibitors of protein and nucleic acid synthesis. Their main effects are on rapidly proliferating tissues such as the bone marrow, skin, mucosal epithelia, and germ cells. In a successful BW attack with trichothecene toxin (T-2), the toxin(s) can adhere to and penetrate the skin, be inhaled, or can be ingested. Clothing would be contaminated and serve as a reservoir for further toxin exposure. Early symptoms beginning within minutes of exposure include burning skin pain, redness, tenderness, blistering, and progression to skin necrosis with leathery blackening and sloughing of large areas of skin in lethal cases. Nasal contact is

manifested by nasal itching and pain, sneezing, epistaxis and rhinorrhea; pulmonary/tracheobronchial toxicity by dyspnea, wheezing, and cough; and mouth and throat exposure by pain and blood tinged saliva and sputum. Anorexia, nausea, vomiting and watery or bloody diarrhea with abdominal crampy pain occurs with gastrointestinal toxicity. Eye pain, tearing, redness, foreign body sensation and blurred vision may follow entry of toxin into the eyes. Skin symptoms occur in minutes to hours and eye symptoms in minutes. Systemic toxicity is manifested by weakness, prostration, dizziness, ataxia, and loss of coordination. Tachycardia, hypothermia, and hypotension follow in fatal cases. Death may occur in minutes, hours or days. The most common symptoms are vomiting, diarrhea, skin involvement with burning pain, redness and pruritus, rash or blisters, bleeding, and dyspnea.

Diagnosis

Rapid onset of symptoms in minutes to hours supports a diagnosis of a chemical or toxin attack. Mustard agents must be considered but they have an odor, are visible, and can be rapidly detected by a field available chemical test. Symptoms from mustard toxicity are also delayed for several hours after which mustard can cause skin, eye and respiratory symptoms. Staphylococcal enterotoxin B delivered by an aerosol attack can cause fever, cough, dyspnea and wheezing but does not involve the skin and eyes. Nausea, vomiting, and diarrhea may follow swallowing of inhaled toxin. Ricin inhalation can cause severe respiratory distress, cough, nausea and arthralgias. Swallowed agent can cause vomiting, diarrhea, and gastrointestinal bleeding, but it spares the skin, nose and eyes. Specific diagnosis of T-2 mycotoxins in the form of a rapid diagnostic test is not presently available in the field. Removal of blood, tissue from fatal cases, and environmental samples for testing using a gas liquid chromatography-mass spectrometry technique will confirm the toxic exposure. This system can detect as little as 0.1-1.0 ppb of T-2. This degree of sensitivity is capable of measuring T-2 levels in the plasma of toxin victims.

Medical Management

Use of a chemical protective mask and clothing prior to and during a mycotoxin aerosol attack will prevent illness. If a soldier is unprotected during an attack the outer uniform should be removed within 4 hours and decontaminated by exposure to 5% hypochlorite for 6-10 hours. The skin should be thoroughly washed with soap and uncontaminated water if available. The M291 skin decontamination kit should also be used to remove skin adherent T-2. Superactivated charcoal can absorb swallowed T-2 and should be administered to victims of an unprotected aerosol attack. The eyes should be irrigated with normal saline or water to remove toxin. No specific antidote or therapeutic regimen is currently available. All therapy is supportive.

PROPHYLAXIS

Physical protection of the skin and airway are the only proven effective methods of protection during an attack. Immunological (vaccines) and chemoprotective pretreatments are being studied in animal models, but are not available for field use by the warfighter.

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DETECTION

Adequate and accurate intelligence is required in order to develop an effective defense against biological warfare. Once an agent has been dispersed, detection of the biological aerosol prior to its arrival over the target, in time for personnel to don protective equipment, is the best way to minimize or prevent casualties. However, interim systems of detecting biological agents are just now being fielded in limited numbers. Until reliable detectors are available in sufficient numbers, usually the first indication of a biological attack in unprotected soldiers will be the ill soldier.

Detector systems are evolving, and represent an area of intense interest with the highest priorities within the research and development community. Several systems are now being fielded. The Biological Integrated Detection System (BIDS) is vehicle mounted and concentrates aerosol particles from environmental air, then subjects the particle sample to both generic and antibody-based detection schemes for selected agents. The Long Range Standoff Detection System (LRSDS) will provide a first time biological standoff detection capability to provide early warning. It will employ infrared laser to detect aerosol clouds at a standoff distance up to 30 kilometers. An improved version is in development to extend the range to 100 km. This system will be available for fixed-site applications or inserted into various transport platforms such as fixed-wing or rotary aircraft. In the research and development phase is the Short-Range Biological Standoff Detection System (SRBSDS). It will employ an ultraviolet and laser-induced fluorescence to detect biological aerosol clouds at distances up to 5 kilometers. The information will be used to provide early warning, enhance contamination avoidance efforts, and cue other detection efforts.

The principal difficulty in detecting biological agent aerosols stems from differentiating the artificially generated BW cloud from the background of organic matter normally present in the atmosphere. Therefore, the aforementioned detection methods must be used in conjunction with medical protection (vaccines and other chemoprophylactic measures), intelligence, and physical protection to provide layered primary defenses against a biological attack.

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PERSONAL PROTECTION

The currently fielded chemical protective equipment, which includes the protective mask, battle dress overgarment (BDO), protective gloves, and overboots will provide protection against a biological agent attack.

The M40 protective mask is available in three sizes, and when worn correctly, will protect the face, eyes, and respiratory tract. The M40 utilizes a single screw on filter element which involves two separate but complementary mechanisms: 1) impaction and adsorption of agent molecules onto ASC Whetlerite Carbon filtration media, and 2) static electrical attraction of particles initially failing to contact the filtration media. Proper maintenance and periodic replacement of the crucial filter elements are of the utmost priority. The filter MUST be replaced under these circumstances: the elements are immersed in water, crushed, cut, or otherwise damaged; excessive breathing resistance is encountered; the "ALL CLEAR" signal is given after exposure to a biological agent; 30 days have elapsed in the combat theater of operations (the filters must be replaced every 30 days); supply bulletins indicate lot number expiration; or when ordered by the unit commander. The filter element can only be changed in a non-contaminated environment. Two styles of optical inserts for the protective mask are available for soldiers requiring visual correction. The wire frame style is considered to be the safer of the two and is more easily fitted into the mask. A prong-type optical insert is also available. A drinking tube on the mask allows the wearer to drink while in a contaminated environment. Note that the wearer should disinfect the canteen and tube by wiping with a 5 percent hypochlorite solution before use.

The battle dress overgarment suits come in eight sizes and are currently available in both woodland and desert camouflage patterns. The suit may be worn for 24 continuous hours in a contaminated environment, but once contaminated, it must be replaced by using the MOPP-gear exchange procedure described in the Soldier's Manual of Common Tasks. The discarded BDO must be incinerated or buried. Chemical protective gloves and overboots come in various sizes and are both made from butyl rubber. They may be decontaminated and reissued. The gloves and overboots must be visually inspected and decontaminated as needed after every 12 hours of exposure in a contaminated environment. While the protective equipment will protect against biological agents, it is important to note that even standard uniform clothing of good quality affords a reasonable protection against dermal exposure of surfaces covered.

Those casualties unable to continue wearing protective equipment should be held and/or transported within casualty wraps designed to protect the patient against further chemical-biological agent exposure. Addition of a filter blower unit to provide overpressure enhances protection and provides cooling.

Collective protection by the use of either a hardened or unhardened shelter equipped with an air filtration unit providing overpressure can offer protection for personnel in the biologically contaminated environment. An airlock ensures that no contamination will be brought into the shelter. In the absence of a dedicated structure, enhanced protection can be afforded within most buildings by sealing cracks and entry ports, and providing air filtration with high efficiency particulate air (HEPA) filters within existing ventilation systems. The key problem is that these shelters can be very limited in military situations, very costly to produce and maintain, and difficult to deploy. Personnel must be decontaminated prior to entering the collective protection unit.

The most important route of exposure to biological agents is through inhalation. Biological warfare (BW) agents are dispersed as aerosols by one of two basic mechanisms: point or line source dissemination. Unlike some chemical threats, aerosols of agents disseminated by line source munitions (e.g., sprayed by low-flying aircraft or speedboats along the coast) do not leave hazardous environmental residua (although anthrax spores may persist and could pose a hazard near the dissemination line). On the other hand, aerosols generated by point-source munitions (i.e., stationary aerosol generator, bomblets, etc.) are more apt to produce ground contamination, but only in the immediate vicinity of dissemination. Point-source munitions leave an obvious signature that alerts the field commander that a biological warfare attack has occurred. Because point-source munitions always leave an agent residue, this evidence can be exploited for detection and identification purposes.

Aerosol delivery systems for biological warfare agents most commonly generate invisible clouds with particles or droplets of < 10 micrometers (μ m). They can remain suspended for extensive periods. The major risk is pulmonary retention of inhaled particles. To a much lesser extent, particles may adhere to an individual or his clothing, thus the need for individual decontamination. The effective area covered varies with many factors, including wind speed, humidity, and sunlight. In the absence of an effective real-time alarm system or direct observation of an attack, the first clue would be mass casualties fitting a clinical pattern compatible with one of the biological agents. This may occur hours or days after the attack.

Toxins may cause direct pulmonary toxicity or be absorbed and cause systemic toxicity. Toxins are frequently as potent or more potent by inhalation than by any other route. A unique clinical picture may sometimes be seen which is not observed by other routes (e.g., pulmonary edema after staphylococcal enterotoxin B (SEB) exposure). Mucous membranes, including conjunctivae, are also vulnerable to many biological warfare agents. Physical protection is then quite important and the use of full-face masks equipped with small-particle filters, like the chemical protective masks, assumes a high degree of importance.

Other routes for delivery of biological agents are thought to be less important than inhalation, but are nonetheless potentially significant. Contamination of food and water supplies, either purposefully or incidentally after an aerosol biological warfare attack, represents a hazard for infection or intoxication by ingestion. Assurance that food and

water supplies are free from contamination should be provided by appropriate preventive medicine authorities in the event of an attack.

Intact skin provides an excellent barrier for most biological agents. T-2 mycotoxins would be an exception because of their dermal activity. However, mucous membranes and abraded, or otherwise damaged, integument can allow for passage of some bacteria and toxins, and should be protected in the event of an attack.

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DECONTAMINATION

Contamination is the introduction of microorganisms into tissues or sterile materials. Decontamination is disinfection or sterilization of infected articles to make them suitable for use (the reduction of microorganisms to an acceptable level). Disinfection is the selective elimination of certain undesirable microorganisms in order to prevent their transmission (the reduction of the number of infectious organisms below the level necessary to cause infection). Sterilization is the complete killing of all organisms. BW agents can be decontaminated by mechanical, chemical and physical methods.

Decontamination methods have always played an important role in the control of infectious diseases. However, we are often unable use the most efficient means of rendering infectious diseases harmless (e.g., toxic chemical sterilization) in order to not hurt people or damage materials which are to be freed from contamination.

Mechanical decontamination involves measures to remove but not necessarily neutralize an agent. An example is the filtering of drinking water to remove certain agents (e.g., V*ibrio cholera* or *Clostridium botulinum*) that may have been used to purposefully contaminate a water supply.

Chemical decontamination renders BW agents harmless by the use of disinfectants which are usually in the form of a liquid, gas or aerosol. One has to remember that some disinfectants are harmful to humans, animals, the environment, and/or materials.

Dermal exposure with a suspected BW agent should be immediately treated by soap and water decontamination. Careful washing with soap and water removes a very large amount of the agent from the skin surface. It is important to use a brush to ensure mechanical loosening from the skin surface structures, and then rinse with copious amounts of water. This method is often sufficient to avert contact infection. The contaminated areas should then be washed with a 0.5% sodium hypochlorite solution, if available, with a contact time of 10 to 15 minutes.

Ampules of calcium hypochlorite (HTH) are also currently fielded in the Chemical Agent Decon Set for mixing hypochlorite solutions. The 0.5% solution can be made by adding one 6-ounce container of calcium hypochlorite to five gallons of water. The 5% solution

can be made by adding eight 6-ounce ampules of calcium hypochlorite to five gallons of water. These solutions evaporate quickly at high temperatures so if they are made in advance they should be stored in closed containers. Also the chlorine solutions should be placed in distinctly marked containers because it is very difficult to tell the difference between the 5% chlorine solution and the 0.5% solution.

To mix a 0.5% sodium hypochlorite solution, take one part Clorox and nine parts water (1:9) since standard stock Clorox is a 5.25% sodium hypochlorite solution. The solution is then applied with a cloth or swab. The solution should be made fresh daily with the pH in the alkaline range.

Chlorine solution must NOT be used in patients with (1) open abdominal wounds, as it may lead to the formation of adhesions, or (2) brain and spinal cord injuries. However, this solution may be instilled into non-cavity wounds and then removed by suction to an appropriate disposal container. Within about 5 minutes, this contaminated solution will be neutralized and nonhazardous. Subsequent irrigation with saline or other surgical solutions should be performed. Prevent the chlorine solution from being sprayed into the eyes, as corneal opacities may result.

For decontamination of fabric clothing or equipment, a 5% hypochlorite solution should be used. For decontamination of equipment, a contact time of 30 minutes prior to normal cleaning is required. This is corrosive to most metals and injurious to most fabrics, so rinse thoroughly and oil metal surfaces after completion.

BW agents can be rendered harmless through such physical means as heat and radiation. To render agents completely harmless, sterilize with dry heat for two hours at 160 degrees centigrade. If autoclaving with steam at 121 degrees centigrade and 1 atmosphere of overpressure (15 pounds per square inch), the time may be reduced to 20 minutes, depending on volume. Solar ultraviolet radiation (UV radiation) has a certain disinfectant effect, often in combination with drying. This is effective in certain environmental conditions but hard to standardize for practical usage for decontamination purposes.

Rooms in fixed spaces are best decontaminated with gases or liquids in aerosol form (e.g., formaldehyde). This is usually combined with surface disinfectants to ensure complete decontamination. Environmental decontamination of terrain is costly and difficult and should be avoided, if possible. If contaminated terrain, streets, or roads must be passed, spray with a dust-binding spray to minimize reaerosolization. If it is necessary to decontaminate these surfaces, chlorine-calcium or lye may be used. Otherwise, rely on the natural processes which, especially outdoors, leads to the decontamination of agent by means of drying and solar UV radiation.

Appendix A: Glossary of Medical Terms

Adapted from Stedman's Electronic Medical Dictionary,

Williams & Wilkins, Baltimore, MD, 1996 and

Principles and Practice of Infectious Diseases,

Mandell et al, Third Edition.

Acetylcholine (ACH, Ach) - The neurotransmitter substance at cholinergic synapses, which causes cardiac inhibition, vasodilation, gastrointestinal peristalsis, and other parasympathetic effects. It is liberated from preganglionic and postganglionic endings of parasympathetic fibers and from preganglionic fibers of the sympathetic as a result of nerve injuries, whereupon it acts as a transmitter on the effector organ; it is hydrolyzed into choline and acetic acid by acetylcholinesterase before a second impulse may be transmitted.

Active immunization -The act of artificially stimulating the body to develop antibodies against infectious disease by the administration of vaccines or toxoids.

Adenopathy - Swelling or morbid enlargement of the lymph nodes.

Aleukia - Absence or extremely decreased number of leukocytes in the circulating blood.

Analgesic - 1. A compound capable of producing analgesia, i.e., one that relieves pain by altering perception of nociceptive stimuli without producing anesthesia or loss of consciousness. 2. Characterized by reduced response to painful stimuli.

Anaphylaxis - The term is commonly used to denote the immediate, transient kind of immunologic (allergic) reaction characterized by contraction of smooth muscle and dilation of capillaries due to release of pharmacologically active substances (histamine, bradykinin, serotonin, and slow-reacting substance), classically initiated by the combination of antigen (allergen) with mast cell-fixed, cytophilic antibody (chiefly IgE).

Anticonvulsant - An agent which prevents or arrests seizures.

Antitoxin - An antibody formed in response to and capable of neutralizing a biological poison.; an animal serum containing antitoxins.

Arthralgia - Severe pain in a joint, especially one not inflammatory in character.

AST - Abbreviation for aspartate aminotransferase, a liver enzyme.

Asthenia - Weakness or debility.

Ataxia - An inability to coordinate muscle activity during voluntary movement, so that smooth movements occur. Most often due to disorders of the cerebellum or the posterior columns of the spinal cord; may involve the limbs, head, or trunk.

Atelectasis - Absence of gas from a part or the whole of the lungs, due to failure of expansion or resorption of gas from the alveoli.

Atropine - An anticholinergic, with diverse effects (tachycardia, mydriasis, cycloplegia, constipation, urinary retention) attributable to reversible competitive blockade of acetylcholine at muscarinic type cholinergic receptors; used in the treatment of poisoning with organophosphate insecticides or nerve gases.

Bilirubin - A red bile pigment formed from hemoglobin during normal and abnormal destruction of erythrocytes. Excess bilirubin is associated with jaundice.

Blood agar - A mixture of blood and nutrient agar, used for the cultivation of many medically important microorganisms.

Bronchiolitis - Inflammation of the bronchioles, often associated with bronchopneumonia.

Bronchitis - Inflammation of the mucous membrane of the bronchial tubes.

Brucella - A genus of encapsulated, nonmotile bacteria (family Brucellaceae) containing short, rod-shaped to coccoid, Gram-negative cells. These organisms are parasitic, invading all animal tissues and causing infection of the genital organs, the mammary gland, and the respiratory and intestinal tracts, and are pathogenic for man and various species of domestic animals. They do not produce gas from carbohydrates.

Bubo - Inflammatory swelling of one or more lymph nodes, usually in the groin; the confluent mass of nodes usually suppurates and drains pus.

Bulla, gen. and pl. bullae - A large blister appearing as a circumscribed area of separation of the epidermis from the subepidermal structure (subepidermal *bulla*) or as a circumscribed area of separation of epidermal cells (intraepidermal *bulla*) caused by the presence of serum, or occasionally by an injected substance.

Carbuncle - Deep-seated pyogenic infection of the skin and subcutaneous tissues, usually arising in several contiguous hair follicles, with formation of connecting sinuses; often preceded or accompanied by fever, malaise, and prostration.

Cerebrospinal - Relating to the brain and the spinal cord.

Chemoprophylaxis - Prevention of disease by the use of chemicals or drugs.

Cholinergic - Relating to nerve cells or fibers that employ acetylcholine as their neurotransmitter.

CNS - Abbreviation for central nervous system.

Coagulopathy - A disease affecting the coagulability of the blood.

Coccobacillus - A short, thick bacterial rod of the shape of an oval or slightly elongated coccus.

Conjunctiva, pl. conjunctivae - The mucous membrane investing the anterior surface of the eyeball and the posterior surface of the lids.

CSF - Abbreviation for cerebrospinal fluid.

Cutaneous - Relating to the skin.

Cyanosis - A dark bluish or purplish coloration of the skin and mucous membrane due to deficient oxygenation of the blood, evident when reduced hemoglobin in the blood exceeds 5 g per 100 ml.

Diathesis -The constitutional or inborn state disposing to a disease, group of diseases, or metabolic or structural anomaly.

Diplopia -The condition in which a single object is perceived as two objects.

Distal - Situated away from the center of the body, or from the point of origin; specifically applied to the extremity or distant part of a limb or organ.

Dysarthria - A disturbance of speech and language due to emotional stress, to brain injury, or to paralysis, incoordination, or spasticity of the muscles used for speaking.

Dysphagia, dysphagy - Difficulty in swallowing.

Dysphonia - Altered voice production.

Dyspnea - Shortness of breath, a subjective difficulty or distress in breathing, usually associated with disease of the heart or lungs; occurs normally during intense physical exertion or at high altitude.

Ecchymosis - A purplish patch caused by extravasation of blood into the skin, differing from petechiae only in size (larger than 3 mm diameter).

Eczema - Generic term for inflammatory conditions of the skin, particularly with vesiculation in the acute stage, typically erythematous, edematous, papular, and crusting; followed often by lichenification and scaling and occasionally by duskiness of

the erythema and, infrequently, hyperpigmentation; often accompanied by sensations of itching and burning.

Edema - An accumulation of an excessive amount of watery fluid in cells, tissues, or serous cavities.

Enanthem, enanthema - A mucous membrane eruption, especially one occurring in connection with one of the exanthemas.

Encephalitis, pl. encephalitides - Inflammation of the brain.

Endotoxemia - Presence in the blood of endotoxins.

Endotracheal intubation - Passage of a tube through the nose or mouth into the trachea for maintenance of the airway during anesthesia or for maintenance of an imperiled airway.

Enterotoxin - A cytotoxin specific for the cells of the intestinal mucosa.

Epistaxis - Profuse bleeding from the nose.

Epizootic - 1. Denoting a temporal pattern of disease occurrence in an animal population in which the disease occurs with a frequency clearly in excess of the expected frequency in that population during a given time interval.

2. An outbreak (epidemic) of disease in an animal population; often with the implication that it may also affect human populations.

Erythema - Redness of the skin due to capillary dilatation.

Erythema multiforme - An acute eruption of macules, papules, or subdermal vesicles presenting a multiform appearance, the characteristic lesion being the target or iris lesion over the dorsal aspect of the hands and forearms; its origin may be allergic, seasonal, or from drug sensitivity, and the eruption, although usually self-limited (e.g., multiforme minor), may be recurrent or may run a severe course, sometimes with fatal termination (e.g., multiforme major or Stevens-Johnson syndrome).

Erythrocyte - A mature red blood cell.

Erythropoiesis - The formation of red blood cells.

Exanthema - A skin eruption occurring as a symptom of an acute viral or coccal disease, as in scarlet fever or measles.

Extracellular -Outside the cells.

Extraocular - Adjacent to but outside the eyeball.

Fasciculation - Involuntary contractions, or twitchings, of groups (fasciculi) of muscle fibers, a coarser form of muscular contraction than fibrillation.

Febrile - Denoting or relating to fever.

Fomite - Objects, such as clothing, towels, and utensils that possibly harbor a disease agent and are capable of transmitting it.

Formalin - A 37% aqueous solution of formaldehyde.

Fulminant hepatitis - Severe, rapidly progressive loss of hepatic function due to viral infection or other cause of inflammatory destruction of liver tissue.

Generalized vaccinia - Secondary lesions of the skin following vaccination which may occur in subjects with previously healthy skin but are more common in the case of traumatized skin, especially in the case of eczema (eczema vaccinatum). In the latter instance, generalized vaccinia may result from mere contact with a vaccinated person. Secondary vaccinial lesions may also occur following transfer of virus from the vaccination to another site by means of the fingers (autoinnoculation).

Glanders - A chronic debilitating disease of horses and other equids, as well as some members of the cat family, caused by *Pseudomonas mallei*; it is transmissible to humans. It attacks the mucous membranes of the nostrils of the horse, producing an increased and vitiated secretion and discharge of mucus, and enlargement and induration of the glands of the lower jaw.

Granulocytopenia -Less than the normal number of granular leukocytes in the blood.

Guarnieri bodies - Intracytoplasmic acidophilic inclusion body's observed in epithelial cells in variola (smallpox) and vaccinia infections, and which include aggregations of Paschen body's or virus particles.

Hemagglutination - The agglutination of red blood cells; may be immune as a result of specific antibody either for red blood cell antigens per se or other antigens which coat the red blood cells, or may be nonimmune as in hemagglutination caused by viruses or other microbes.

Hemagglutinin - A substance, antibody or other, that causes hemagglutination.

Hematemesis - Vomiting of blood.

Hemopoietic - Pertaining to or related to the formation of blood cells.

Hematuria - Any condition in which the urine contains blood or red blood cells.

Hemodynamic - Relating to the physical aspects of the blood circulation.

Hemolysis -Alteration, dissolution, or destruction of red blood cells in such a manner that hemoglobin is liberated into the medium in which the cells are suspended, e.g., by specific complement-fixing antibodies, toxins, various chemical agents, tonicity, alteration of temperature.

Hemolytic Uremic Syndrome - Hemolytic anemia and thrombocytopenia occurring with acute renal failure.

Hemoptysis - The spitting of blood derived from the lungs or bronchial tubes as a result of pulmonary or bronchial hemorrhage.

Hepatic - Relating to the liver.

Heterologous - 1. Pertaining to cytologic or histologic elements occurring where they are not normally found.

2. Derived from an animal of a different species, as the serum of a horse is heterologous for a rabbit.

Hyperemia - The presence of an increased amount of blood in a part or organ.

Hyperesthesia - Abnormal acuteness of sensitivity to touch, pain, or other sensory stimuli.

Hypotension - Subnormal arterial blood pressure.

Hypovolemia - A decreased amount of blood in the body.

Hypoxemia - Subnormal oxygenation of arterial blood, short of anoxia.

Idiopathic - Denoting a disease of unknown cause.

Immunoassay - Detection and assay of substances by serological (immunological) methods; in most applications the substance in question serves as antigen, both in antibody production and in measurement of antibody by the test substance.

In vitro - In an artificial environment, referring to a process or reaction occurring therein, as in a test tube or culture media.

In vivo - In the living body, referring to a process or reaction occurring therein.

Induration - 1. The process of becoming extremely firm or hard, or having such physical features.

2. A focus or region of indurated tissue.

Inguinal - Relating to the groin.

Inoculation - Introduction into the body of the causative organism of a disease.

Leukopenia - The antithesis of leukocytosis; any situation in which the total number of leukocytes in the circulating blood is less than normal, the lower limit of which is generally regarded as 4000-5000 per cu mm.

Lumbosacral - Relating to the lumbar vertebrae and the sacrum.

Lumen, pl. lumina - The space in the interior of a tubular structure, such as an artery or the intestine.

Lymphadenopathy - Any disease process affecting a lymph node or lymph nodes.

Lymphopenia - A reduction, relative or absolute, in the number of lymphocytes in the circulating blood.

Macula, pl. maculae - 1. A small spot, perceptibly different in color from the surrounding tissue. 2. A small, discolored patch or spot on the skin, neither elevated above nor depressed below the skin's surface.

Mediastinitis - Inflammation of the cellular tissue of the mediastinum.

Mediastinum - The median partition of the thoracic cavity, covered by the mediastinal pleura and containing all the thoracic viscera and structures except the lungs.

Megakaryocyte - A large cell with a polyploid nucleus that is usually multilobed; megakaryocytes are normally present in bone marrow, not in the circulating blood, and give rise to blood platelets.

Melena - Passage of dark-colored, tarry stools, due to the presence of blood altered by the intestinal juices.

Meningism - A condition in which the symptoms simulate a meningitis, but in which no actual inflammation of these membranes is present.

Meningococcemia - Presence of meningococci (*N. meningitidis*) in the circulating blood.

Meninges - Any membrane; specifically, one of the membranous coverings of the brain and spinal cord.

Microcyst - A tiny cyst, frequently of such dimensions that a magnifying lens or microscope is required for observation.

Microscopy - Investigation of minute objects by means of a microscope.

Moribund - Dying; at the point of death.

Mucocutaneous - Relating to mucous membrane and skin; denoting the line of junction of the two at the nasal, oral, vaginal, and anal orifices.

Myalgia - Muscular pain.

Mydriasis - Dilation of the pupil.

Narcosis - General and nonspecific reversible depression of neuronal excitability, produced by a number of physical and chemical agents, usually resulting in stupor rather than in anesthesia.

Necrosis - Pathologic death of one or more cells, or of a portion of tissue or organ, resulting from irreversible damage.

Nephropathia epidemica - A generally benign form of epidemic hemorrhagic fever reported in Scandinavia.

Neutrophilia - An increase of neutrophilic leukocytes in blood or tissues; also frequently used synonymously with leukocytosis, inasmuch as the latter is generally the result of an increased number of neutrophilic granulocytes in the circulating blood (or in the tissues, or both).

Nosocomial - Denoting a new disorder (not the patient's original condition) associated with being treated in a hospital, such as a hospital-acquired infection.

Oliguria - Scanty urine production.

Oropharynx - The portion of the pharynx that lies posterior to the mouth; it is continuous above with the nasopharynx via the pharyngeal isthmus and below with the laryngopharynx.

Osteomyelitis - Inflammation of the bone marrow and adjacent bone.

Pancytopenia - Pronounced reduction in the number of erythrocytes, all types of white blood cells, and the blood platelets in the circulating blood.

Pandemic - Denoting a disease affecting or attacking the population of an extensive region, country, continent; extensively epidemic.

Papule - A small, circumscribed, solid elevation on the skin.

Parasitemia -The presence of parasites in the circulating blood; used especially with reference to malarial and other protozoan forms, and microfilariae.

Passive immunity - Providing temporary protection from disease through the administration of exogenously produced antibody (i.e., transplacental transmission of antibodies to the fetus or the injection of immune globulin for specific preventive purposes).

PCR - see below for polymerase chain reaction.

Percutaneous - Denoting the passage of substances through unbroken skin, for example, by needle puncture, including introduction of wires and catheters.

Perivascular - Surrounding a blood or lymph vessel.

Petechia, pl. petechiae - Minute hemorrhagic spots, of pinpoint to pinhead size, in the skin, which are not blanched by pressure.

Pharyngeal - Relating to the pharynx.

Pharyngitis - Inflammation of the mucous membrane and underlying parts of the pharynx.

Phosgene - Carbonyl chloride; a colorless liquid below 8.2°C, but an extremely poisonous gas at ordinary temperatures; it is an insidious gas, since it is not immediately irritating, even when fatal concentrations are inhaled.

Photophobia - Morbid dread and avoidance of light. Photosensitivity, or pain in the eyes with exposure to light, can be a cause.

Pleurisy - Inflammation of the pleura.

Polymerase chain reaction - An in vitro method for enzymatically synthesizing and amplifying defined sequences of DNA in molecular biology. Can be used for improving DNA-based diagnostic procedures for identifying unknown BW agents.

Polymorphonuclear - Having nuclei of varied forms; denoting a variety of leukocyte.

Polyuria - Excessive excretion of urine.

Presynaptic - Pertaining to the area on the proximal side of a synaptic cleft.

Prophylaxis, pl. prophylaxes - Prevention of disease or of a process that can lead to disease.

Prostration - A marked loss of strength, as in exhaustion.

Proteinuria - Presence of urinary protein in concentrations greater than 0.3 g in a 24-hour urine collection or in concentrations greater than 1 g/l in a random urine collection on two or more occasions at least 6 hours apart; specimens must be clean, voided midstream, or obtained by catheterization.

Pruritus - Syn: itching.

Ptosis, pl. ptoses - In reference to the eyes, drooping of the eyelids.

Pulmonary edema -Edema of the lungs.

Pyrogenic - Causing fever.

Retinitis - Inflammation of the retina.

Retrosternal - Posterior to the sternum.

Rhinorrhea - A discharge from the nasal mucous membrane.

Sarin - A nerve poison which is a very potent irreversible cholinesterase inhibitor and a more toxic nerve gas than tabun or soman.

Scarification -The making of a number of superficial incisions in the skin. It is the technique used to administer tularemia and smallpox vaccines.

Septic shock - 1. shock associated with sepsis, usually associated with abdominal and pelvic infection complicating trauma or operations; 2. shock associated with septicemia caused by Gram-negative bacteria.

Sequela, pl. sequelae - A condition following as a consequence of a disease.

Shigellosis - Bacillary dysentery caused by bacteria of the genus *Shigella*, often occurring in epidemic patterns.

Soman - An extremely potent cholinesterase inhibitor, similar to sarin and tabun.

Sterile abscess - An abscess whose contents are not caused by pyogenic bacteria.

Stridor - A high-pitched, noisy respiration, like the blowing of the wind; a sign of respiratory obstruction, especially in the trachea or larynx.

Superantigen - An antigen that interacts with the T cell receptor in a domain outside of the antigen recognition site. This type of interaction induces the activation of larger numbers of T cells compared to antigens that are presented in the antigen recognition site.

Superinfection - A new infection in addition to one already present.

Tachycardia - Rapid beating of the heart, conventionally applied to rates over 100 per minute.

Teratogenicity -The property or capability of producing fetal malformation.

Thrombocytopenia - A condition in which there is an abnormally small number of platelets in the circulating blood.

Toxoid - A modified bacterial toxin that has been rendered nontoxic (commonly with formaldehyde) but retains the ability to stimulate the formation of antitoxins (antibodies) and thus producing an active immunity. Examples include Botulinum, tetanus, and diphtheria toxoids.

Tracheitis - Inflammation of the lining membrane of the trachea.

Urticaria - An eruption of itching wheals, usually of systemic origin; it may be due to a state of hypersensitivity to foods or drugs, foci of infection, physical agents (heat, cold, light, friction), or psychic stimuli.

Vaccine - A suspension of attenuated live or killed microorganisms (bacteria, viruses, or rickettsiae), or fractions thereof, administered to induce immunity and thereby prevent infectious disease.

Vaccinia - An infection, primarily local and limited to the site of inoculation, induced in man by inoculation with the vaccinia (coxpox) virus in order to confer resistance to smallpox (variola). On about the third day after vaccination, papules form at the site of inoculation which become transformed into umbilicated vesicles and later pustules; they then dry up, and the scab falls off on about the 21st day, leaving a pitted scar; in some cases there are more or less marked constitutional disturbances.

Varicella - An acute contagious disease, usually occurring in children, caused by the varicella-zoster virus, a member of the family *Herpesviridae*, and marked by a sparse

eruption of papules, which become vesicles and then pustules, like that of smallpox although less severe and varying in stages, usually with mild constitutional symptoms; incubation period is about 14 to 17 days. Syn: chickenpox

Variola - Syn: smallpox.

Variolation - The historical practice of inducing immunity against smallpox by "scratching" the skin with the purulency from smallpox skin pustules. The first inoculation for smallpox is said to have been done in China about 1022 B.C.

Viremia - The presence of virus in the bloodstream.

Virion - The complete virus particle that is structurally intact and infectious.

Zoonosis - An infection or infestation shared in nature by humans and other animals that are the normal or usual host; a disease of humans acquired from an animal source.

Appendix B: Patient Isolation Precautions

Standard Precautions

- Handwashing after patient contact.
- Use of gloves when touching blood, body fluids, secretions, excretions and contaminated items.
- Use of mask, eye protection, and gown during procedures likely to generate splashes or sprays of blood, body fluids, secretions or excretions
- Handle contaminated patient-care equipment and linen in a manner that prevents the transfer of microorganisms to people or equipment.
- Practice care when handling sharps and use a mouthpiece or other ventilation device as an alternative to mouth-to-mouth resuscitation when practical.
- Place the patient in a private room when feasible if they may contaminate the environment.

Airborne Precautions

Standard Precautions plus:

- Place the patient in a private room that has negative air pressure, at least six air changes/hour, and appropriate filtration of air before it is discharged from the room.
- Use of respiratory protection when entering the room.
- Limit movement and transport of the patient. Use a mask on the patient if they need to be moved.

Droplet Precautions

Standard Precaution plus:

- Place the patient in a private room or with someone with the same infection. If not feasible, maintain at least 3 feet between patients.
- Use of a mask when working within 3 feet of the patient.
- Limit movement and transport of the patient. Use a mask on the patient if they need to be moved.

Appendix B: Patient Isolation Precautions (continued)

Contact Precautions

Standard Precautions plus:

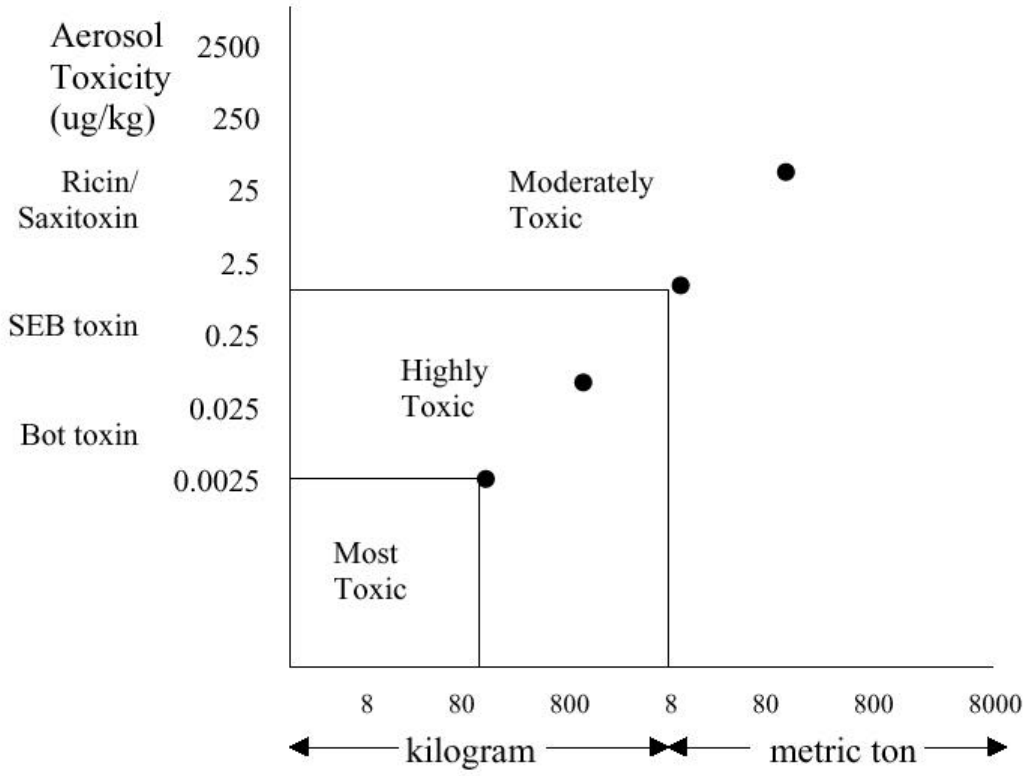
- Place the patient in a private room or with someone with the same infection if possible.
- Use of gloves when entering the room. Change gloves after contact with infective material.
- Use of gown when entering the room if contact with patient is anticipated or if the patient has diarrhea, a colostomy or wound drainage not covered by a dressing.
- Limit the movement or transport of the patient from the room.
- Ensure that patient-care items, bedside equipment, and frequently touched surfaces receive daily cleaning.
- Dedicate use of noncritical patient-care equipment to a single patient, or cohort of patients with the same pathogen. If not feasible, adequate disinfection between patients is necessary.

Appendix C: Comparative Lethality of Selected Toxins & Chemical Agents in Laboratory Mice

AGENT	LD_{50} (μ g/kg)	MOLECULAR WEIGHT	SOURCE
Botulinum toxin	0.001	150,000	Bacterium
Shiga toxin	0.002	55,000	Bacterium
Tetanus toxin	0.002	150,000	Bacterium
Abrin	0.04	65,000	Plant (Rosary Pea)
Diphtheria toxin	0.10	62,000	Bacterium
Maitotoxin	0.10	3,400	Marine Dinoflagellate
Palytoxin	0.15	2,700	Marine Soft Coral
Ciguatoxin	0.40	1,000	Marine Dinoflagellate
Textilotoxin	0.60	80,000	Elapid Snake
C. perfringens toxins	0.1 - 5.0	35 - 40,000	Bacterium
Batrachotoxin	2.0	539	Arrow-Poison Frog
Ricin	3.0	64,000	Plant (Castor Bean)
alpha-Conotoxin	5.0	1,500	Cone Snail
Taipoxin	5.0	46,000	Elapid Snake
Tetrodotoxin	8.0	319	Puffer Fish
alpha-Tityustoxin	9.0	8,000	Scorpion
Saxitoxin	10.0 (Inhal 2.0)	299	Marine Dinoflagellate
VX	15.0	267	Chemical Agent
SEB (Rhesus/Aerosol)	27.0 (ED_{50}~pg)	28,494	Bacterium
Anatoxin-A(s)	50.0	500	Blue-Green Algae
Microcystin	50.0	994	Blue-Green Algae

Soman (GD)	64.0	182	Chemical Agent
Sarin (GB)	100.0	140	Chemical Agent
Aconitine	100.0	647	Plant (Monkshood)
T-2 Toxin	1,210.0	466	Fungal Myotoxin

Appendix D. Aerosol Toxicity in LD50 vs. Quantity of Toxin



Aerosol toxicity in LD50 (see Appendix C) vs. quantity of toxin required to provide a theoretically effective open-air exposure, under ideal meteorological conditions, to an area 100 km 2 . Ricin, saxitoxin and botulinum toxins kill at the concentrations depicted. (Patrick and Spertzel, 1992: Based on Cader K.L., BWL Tech Study #3, Mathematical models for dosage and casualty resulting from single point and line source release of aerosol near ground level, DTIC#AD3 10-361, Dec 1957)

Appendix E: Differential Diagnosis of Chemical Nerve Agent, Botulinum Toxin and SEB Intoxication following Inhalation Exposure

	Chemical Nerve Agent	Botulinum Toxin	SEB
Time to Symptoms	Minutes	Hours (12-48)	Hours (1-6)
Nervous	Convulsions, Muscle twitching	Progressive paralysis	Headache Muscle aches
Cardiovascular	Slow heart rate	Normal rate	Normal or Rapid Heart Rate
Respiratory	Difficult breathing, airways constriction	Normal, then progressive paralysis	Nonproductive cough Severe cases;chest pain/difficult breathing
Gastrointestinal	Increased motility, pain, diarrhea	Decreased motility	Nausea,vomiting and/or diarrhea
Ocular	Small pupils	Droopy eyelids	May see "red eyes" (conjuntival injection)
Salivary	Profuse, watery Saliva	Normal; difficulty swallowing	May be slightly increased quantities of saliva
Death	Minutes	2-3 days	Unlikely
Response to Atropine/2PAM-Cl	Yes	No	Atropine may reduce gastrointestinal symptoms

Appendix F: Specimens for Laboratory Diagnosis

Agent	Face or Nasal Swab[1]	Blood Culture	Smear	Acute & Convalescent Sera	Stool	Urine	Other
Anthrax	+	+	Pleural and CS fluids mediastinal lymph node spleen	+	-	-	Lesion aspirates
Brucellosis	+	+	-	+	-	-	Bone marrow and spinal fluid cultures; tissues, exudates
Cholera	-	-	-	+	+		
Plague	+	+	Sputum	+	-	-	Lymph node, buboes, CSF, and sputum culture
Tularemia	+	-	+	Sputum	+	-	
Q-fever	-	4	Lesions	+			Lung, spleen, lymph cultures
Congo-Crimean Hemorrhagic Fever	-	3	-	+	-	-	Liver
VEE	-	3	-	+	-		CSF
Clostridial Toxins	+		Wound tissue	+	+	-	
SEB Toxin	+	-	-	+	+	+	Lung
Ricin Toxin	+	-	-	+			Spleen, lung, Kidney

[1]Within 18-24 hours
[2]Fluorescent antibody test on infected lymph node smears. Gram stain has little value.
[3]Virus isolation from blood in appropriate containment.
[4]*C. burnetti* can persist for days in blood and resists desiccation. EDTA anticoagulated blood preferred. Culturing should not be done except in BL3 containment.

Appendix G: BW Agent Lab Identification

Agent	**Gold Standard**	**Antigen Capture**	**Immunoassays**		**PCR**	**Animals**
			IgG	**IgM**		
Aflatoxins	Mass spectroscopy					
Alpha Toxin	ELISA	X				
Alphaviruses	Virus isolation/neutralization	X	X	X	X	X
Arboviruses	Virus isolation/IFA	X	X	X	X	X
Bacillus anthracis	IFA/Std. Microbiology	X (PA)	X	X	X	X
Bacillus globigii	Std. Microbiology				X	
Bacillus thuringiensis	Std. Microbiology					
Bot Toxin	Mouse neutralization	X (A Toxin)			*	X
Brucella sp.	IFA/Std. Microbiology	X	X	X	X	X
C. burnetii	IFA/Std. Microbiology/serology				X	X
C. perfringens						
Clostridium sp.	Std. Microbiology					
F. tularensis	IFA/Std. Microbiology	X			X	X
Filoviruses	Virus isolation/neutralization	X	X	X	X	X
Hantaviruses	Virus isolation/IFA	X	X	X	X	X
Orthopox Viruses	Virus isolation/IFA	X			X	X
Ricin Toxin	ELISA	X	X			X
Saxitoxin	Bioassay		(neutralizing antibodies)			X

SEA Toxin					*	
SEB Toxin	ELISA	X			*	X
Shigella sp.	Std. Microbiology					
Tetrodotoxins	Bioassay		(neutralizing antibodies)			X
Vibrio cholerae	Std. Microbiology/serology	X	X	X	X	
Yersinia pestis	IFA/Std. Microbiology	X (F1)	X	X	X	X

*Toxin gene detected

ELISA - enzyme-linked immunosorbent assays

IFA - indirect or direct immunofluorescence assays

Std. Micro./serology - standard microbiological techniques available, including electron microscopy

Appendix H: BW Agent Characteristics

Disease	Transmit Man to Man	Infective Dose (Aerosol)	Incubation Period	Duration of Illness	Lethality	Persistence of Organism	Vaccine Efficacy (aerosol exposure)
Inhalation anthrax	No	8,000-50,000 spores	1-6 days	3-5 days (usually fatal if untreated)	High	Very stable - spores remain viable for > 40 years in soil	2 dose efficacy against 200-500 LD_{50} in monkeys
Brucellosis	No	10 -100 organisms	5-60 days (usually 1-2 months)	Weeks to months	<5% untreated	Very stable	No vaccine
Cholera	Rare	10-500 organisms	4 hours - 5 days (usually 2-3 days)	$\geq$ 1 week	Low with treatment, high without	Unstable in aerosols & fresh water; stable in salt water	No data on aerosol
Glanders	Low	Assumed low	10-14 days via aerosol	Death in 7-10 days in septicemic form	> 50%	Very stable	No vaccine
Pneumonic Plague	High	100-500 organisms	2-3 days	1-6 days (usually fatal)	High unless treated within 12-24 hours	For up to 1 year in soil; 270 days in live tissue	3 doses not protective against 118 LD_{50} in monkeys
Tularemia	No	10-50 organisms	2-10 days (average 3-5)	$\geq$ 2 weeks	Moderate if untreated	For months in moist soil or other media	80% protection against 1-10 LD_{50}
Q Fever	Rare	1-10 organisms	10-40 days	2-14 days	Very low	For months on wood and sand	94% protection against 3,500 LD_{50} in guinea pigs
Smallpox	High	Assumed low (10-100 organisms)	7-17 days (average 12)	4 weeks	High to moderate	Very stable	Vaccine protects against large doses in primates
Venezuelan Equine Encephalitis	Low	10-100 organisms	2-6 days	Days to weeks	Low	Relatively unstable	TC 83 protects against 30-500 LD_{50} in hamsters
Viral Hemorrhagic Fevers	Moderate	1-10 organisms	4-21 days	Death between 7-16 days	High for Zaire strain, moderate with Sudan	Relatively unstable	No vaccine
Botulism	No	0.001 μ g/kg is LD_{50} for type A	1-5 days	Death in 24-72 hours; lasts months if not lethal	High without respiratory support	For weeks in nonmoving water and food	3 dose efficacy 100% against 25-250 LD_{50} in primates
Staph Enterotoxin B	No	0.03 μ g/person incapacitation	3-12 hours after inhalation	Hours	< 1%	Resistant to freezing	No vaccine
Ricin	No	3-5 μ g/kg is LD_{50} in mice	18-24 hours	Days - death within 10-12 days for ingestion	High	Stable	No vaccine
T-2 Mycotoxins	No	Moderate	2-4 hours	Days to months	Moderate	For years at room temperature	No vaccine

Appendix I: BW Agents: Vaccine, Therapeutics, and Prophylaxis

DISEASE	VACCINE	CHEMOTHERAPY (Rx)	CHEMOPROPHYLAXIS (Px)	COMMENTS
Anthrax	Bioport vaccine (licensed) 0.5 mL SC @ 0, 2, 4 wk, 6, 12, 18 mo then annual boosters	Ciprofloxacin 400 mg IV q 8-12 h	Ciprofloxacin 500 mg PO bid x 4 wk If unvaccinated, begin initial doses of vaccine	Potential alternates for Rx: gentamicin, erythromycin, and chloramphenicol
		Doxycycline 200 mg IV, then 100 mg IV q 8-12 h	Doxycycline 100 mg PO bid x 4 wk plus vaccination	
		Penicillin 2 million units IV q 2 h		PCN for sensitive organisms only
Cholera	Wyeth-Ayerst Vaccine 2 doses 0.5 mL IM or SC @ 0, 7-30 days, then boosters Q 6 months	Oral rehydration therapy during period of high fluid loss		Vaccine not recommended for routine protection in endemic areas (50% efficacy, short term)
		Tetracycline 500 mg q 6 h x 3 d		Alternates for Rx: erythromycin,
		Doxycycline 300 mg once, or 100 mg q 12 h x 3 d		trimethoprim and sulfamethoxazole, and furazolidone
		Ciprofloxacin 500 mg q 12 h x 3 d		Quinolones for tetra/doxy resistant strains
		Norfloxacin 400 mg q 12 h x 3 d		
Q Fever	IND 610 - inactivated whole cell vaccine given as single 0.5 ml s.c. injection	Tetracycline 500 mg PO q 6 h x 5-7 d	Tetracycline start 8-12 d post-exposure x 5 d	Currently testing vaccine to determine the necessity of skin testing prior to use.
		Doxycycline 100 mg PO q 12 h x 5-7 d	Doxycycline start 8-12 d post-exposure x 5 d	
Glanders	No vaccine available	Sulfadiazine 100 mg/kg in divided doses x 3 weeks may be effective TMP-SMX may be effective	Post-exposure prophylaxis may be tried with TMP-SMX	No large therapeutic human trials have been conducted owing to the rarity of naturally occurring disease.
Plague	Greer inactivated vaccine (FDA licensed): 1.0 mL IM; 0.2 mL IM 1-3 mo later; 0.2 mL 5-6 mo after dose 2; 0.2 mL boosters @ 6,12, 18 mo after dose 3 then q 1-2 years	Streptomycin 30 mg/kg/d IM in 2 divided doses x 10 d (or gentamicin)	Doxycycline 100 mg PO bid x 7 d or duration of exposure Ciprofloxacin 500 mg PO bid x 7 d	Plague vaccine not protective against aerosol challenge in animal studies
		Doxy 200 mg IV then 100 mg IV bid x 10-14 d	Doxycycline 100 mg PO bid x 7 d Tetracycline 500 mg PO qid x 7 d	Alternate Rx: trimethoprim-sulfamethoxazole
		Chloramphenicol 1 gm IV qid x 10-14 d		Chloramphenicol for plague meningitis

DISEASE	VACCINE	CHEMOTHERAPY (Rx)	CHEMOPROPHYLAXIS (Px)	COMMENTS
Tularemia	IND - Live attenuated vaccine: one dose by scarification	Streptomycin 30 mg/kg IM divided BID x 10-14 d	Doxycyline 100 mg PO bid x 14 d	
		Gentamicin 3-5 mg/kg/d IV x 10-14 d	Tetracycline 500 mg PO QID x 14 d	
Brucellosis	No human vaccine available	Doxycycline 200 mg/d PO plus rifampin 600-900 mg/d PO x 6 wk	Doxycycline and rifampin x 3 wk	Trimethoprim-sulfamethoxazole may be substituted for rifampin; however, relapse may reach 30%
		Ofloxacin 400/rifampin 600 mg/d PO x 6 wks		
Viral encephalitides	VEE DOD TC-83 live attenuated vaccine (IND): 0.5 mL SC x1 dose	Supportive therapy: analgesics and anticonvulsants prn	NA	TC-83 reactogenic in 20% No seroconversion in 20% Only effective against subtypes 1A, 1B, and 1C
	VEE DOD C-84 (formalin inactivated TC-83) (IND): 0.5 mL SC for up to 3 doses			C-84 vaccine used for non-responders to TC-83
	EEE inactivated (IND): 0.5 mL SC at 0 & 28 d			EEE and WEE inactivated vaccines are poorly
	WEE inactivated (IND): 0.5 mL SC at 0, 7, and 28 d			Immunogenic. Multiple immunizations are required
Viral Hemorrhagic Fevers	AHF Candid #1 vaccine (x-protection for BHF) (IND)	Ribavirin (CCHF/arenaviruses) 30 mg/kg IV initial dose 15 mg/kg IV q 6 h x 4 d 7.5 mg/kg IV q 8 h x 6 d	NA	Aggresive supportive care and management of hypotension very important
	RVF inactivated	Passive antibody for AHF, BHF, Lassa fever, and CCHF		

	vaccine (IND)			
Smallpox	**Wyeth calf lymph vaccinia vaccine (licensed): 1 dose by scarification**	**Cidofovir (effective in vitro)**	**Vaccinia immune globulin 0.6 mL/kg IM (within 3 d of exposure, best within 24 h)**	**Pre and post exposure vaccination recommended if > 3 years since last vaccine**
Botulism	**DOD pentavalent toxoid for serotypes A - E (IND): 0.5 ml deep SC @ 0, 2 & 12 wk, then yearly boosters**	**DOD heptavalent equine despeciated antitoxin for serotypes A-G (IND): 1 vial (10 mL) IV**		**Skin test for hypersensitivity before equine antitoxin administration**
		CDC trivalent equine antitoxin for serotypes A, B, E (licensed)		
StaphylococcusEnterotoxin B	**No vaccine available**	**Ventilatory support for inhalation exposure**		
Ricin	**No vaccine available**	**Inhalation: supportive therapy G-I : gastric lavage, superactivated charcoal, cathartics**		
T-2 Mycotoxins	**No vaccine available**		**Decontamination of clothing and skin**	

Appendix J: Medical Sample Collection for Biological Threat Agents

This guide helps determine which clinical samples to collect from individuals exposed to aerosolized biological threat agents. Proper collection of specimens is dependent on the time-frame following exposure. Sample collection is described for "Early post-exposure", "Clinical", and "Convalescent/ Terminal/ Postmortem" time-frames. These time-frames are not rigid and will vary according to the concentration of the agent used, the agent strain, and predisposing health factors of the patient.

- Early post-exposure: when it is known that an individual has been exposed to a bioagent aerosol; aggressively attempt to obtain samples as indicated
- Clinical: samples from those individuals presenting with clinical symptoms
- Convalescent/Terminal/Postmortem: samples taken during convalescence, the terminal stages of infection or toxicosis or postmortem during autopsy

Shipping Samples: Most specimens sent rapidly (less than 24 h) to analytical labs require only blue or wet ice or refrigeration at 2 to 8 C. However, if the time span increases beyond 24 h, contact the USAMRIID "Hot-Line" (1-888-USA-RIID) for other shipping requirements such as shipment on dry-ice or in liquid nitrogen.

Blood samples: Several choices are offered based on availability of the blood collection tubes. Do not send blood in all the tubes listed, but merely choose one. Tiger-top tubes that have been centrifuged are preferred over red-top clot tubes with serum removed from the clot, but the latter will suffice. Blood culture bottles are also preferred over citrated blood for bacterial cultures.

Pathology samples: routinely include liver, lung, spleen, and regional or mesenteric lymph nodes. Additional samples requested are as follows: brain tissue for encephalomyelitis cases (mortality is rare) and the adrenal gland for Ebola (nice to have but not absolutely required).

Appendix J: Medical Sample Collection for Biological Threat Agents
Bacteria and Rickettsia

Early post-exposure	Clinical	Convalescent/ Terminal/Postmortem

Anthrax
Bacillus anthracis

0 – 24 h	24 to 72 h	3 to 10 days
Nasal and throat swabs, induced respiratory secretions for culture, FA, and PCR	Serum (TT, RT) for toxin assays Blood (E, C, H) for PCR. Blood (BC, C) for culture	Serum (TT, RT) for toxin assays Blood (BC, C) for culture. Pathology samples

Plague
Yersinia pestis

0 – 24 h	24 – 72 h	>6 days
Nasal swabs, sputum, induced respiratory secretions for culture, FA, and PCR	Blood (BC, C) and bloody sputum for culture and FA (C), F-1 Antigen assays (TT, RT), PCR (E, C, H)	Serum (TT, RT) for IgM later for IgG . Pathology samples

Tularemia
Francisella tularensis

0 – 24 h	24 – 72 h	>6 days
Nasal swabs, sputum, induced respiratory secretions for culture, FA and PCR	Blood (BC, C) for culture Blood (E, C, H) for PCR Sputum for FA & PCR	Serum (TT, RT) for IgM and later IgG, agglutination titers. Pathology Samples

BC: Blood culture bottle C: Citrated blood (3-ml)	E: EDTA (3-ml) H: Heparin (3-ml)	TT: Tiger-top (5 – 10 ml) RT: Red top if no TT

Appendix J: Medical Sample Collection for Biological Threat Agents *Bacteria and Rickettsia*

Early post-exposure	Clinical	Convalescent/ Terminal/Postmortem
Glanders *Burkholderia mallei*		
0 – 24 h Nasal swabs, sputum, induced respiratory secretions for culture and PCR.	>24 – 72 h Blood (BC, C) for culture Blood (E, C, H) for PCR Sputum & drainage from skin lesions for PCR & culture.	>6 days Blood (BC, C) and tissues for culture. Serum (TT, RT) for immunoassays. Pathology samples.
Brucellosis *Brucella abortus*, *suis, & melitensis*		
0 – 24 h Nasal swabs, sputum, induced respiratory secretions for culture and PCR.	24 – 72 h Blood (BC, C) for culture. Blood (E, C, H) for PCR.	>6 days Blood (BC, C) and tissues for culture. Serum (TT, RT) for immunoassays. Pathology samples
Q-Fever *Coxiella burnetii*		
0 – 24 h Nasal swabs, sputum, induced respiratory secretions for culture and PCR.	2 to 5 days Blood (BC, C) for culture in eggs or mouse inoculation Blood (E, C, H) for PCR.	>6 days Blood (BC, C) for culture in eggs or mouse inoculation Pathology samples.

BC: Blood culture bottle
C: Citrated blood (3-ml)

E: EDTA (3-ml)
H: Heparin (3-ml)

TT: Tiger-top (5 - 10 ml)
RT: Red top if no TT

Appendix J: Medical Sample Collection for Biological Threat Agents *Toxins*

Early post-exposure	Clinical	Convalescent/ Terminal/Postmortem
Botulism Botulinum toxin from *Clostridium botulinum*		
0 – 24 h Nasal swabs, induced respiratory secretions for PCR (contaminating bacterial DNA) and toxin assays. Serum (TT, RT) for toxin assays.	24 to 72 h Nasal swabs, respiratory secretions for PCR (contaminating bacterial DNA) and toxin assays.	>6 days Usually no IgM or IgG Pathology samples (liver and spleen for toxin detection)
Ricin Intoxication **Ricin toxin from Castor beans**		
0 – 24 h Nasal swabs, induced respiratory secretions for PCR (contaminating castor bean DNA) and toxin assays. Serum (TT) for toxin assays	36 to 48 h Serum (TT, RT) for toxin assay Tissues for immunohisto-logical stain in pathology samples.	>6 days Serum (TT, RT) for IgM and IgG in survivors
Staph enterotoxicosis *Staphylococcus* Enterotoxin B		
0 – 3 h Nasal swabs, induced respiratory secretions for PCR (contaminating bacterial DNA) and toxin assays. Serum (TT, RT) for toxin assays	- 6 h Urine for immunoassays Nasal swabs, induced respiratory secretions for PCR (contaminating bacterial DNA) and toxin assays. Serum (TT, RT) for toxin assays	>6 days Serum for IgM and IgG

Appendix J: Medical Sample Collection for Biological Threat Agents *Viruses*

Early post-exposure	Clinical	Convalescent/ Terminal/Postmortem
Equine Encephalmyelitis VEE, EEE and WEE viruses		
0 – 24 h Nasal swabs & induced respiratory secretions for RT-PCR and viral culture	24 to 72 h | Serum & Throat swabs for culture (TT, RT), RT-PCR (E, C, H, TT, RT) and Antigen ELISA (TT, RT), CSF, Throat swabs up to 5 days	>6 days Serum (TT, RT) for IgM Pathology samples plus brain
Ebola		
0 – 24 h Nasal swabs & induced respiratory secretions for RT-PCR and viral culture	2 to 5 days Serum (TT, RT) for viral culture	>6 days Serum (TT, RT) for viral culture. Pathology samples plus adrenal gland.
Pox (Small pox, monkey pox) Orthopoxvirus		
0 – 24 h Nasal swabs & induced respiratory secretions for PCR and viral culture	2 to 5 days Serum (TT, RT) for viral culture	>6 days Serum (TT, RT) for viral culture. Drainage from skin lesions/ scrapings for microscopy, EM, viral culture, PCR. Pathology samples

BC: Blood culture bottle
C: Citratd blood (3-ml)

E: EDTA (3-ml)
H: Heparin (3-ml)

TT: Tiger-top (5 - 10 ml)
RT: Red top if no TT